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LINDAU–VON HIPPEL DISEASE WITH HEMANGIOBLASTOMA OF THE SPINAL CORD AND SYRINGOMYELIA

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IN 1926 LINDAU [1] drew attention to the frequency with which angioblastic tumors of the retinas and single or multiple angioblastoma of the central nervous system were associated with visceral defects. Hemangioblastoma of the spinal cord was present in 4 of the 15 cases originally reported by Lindau, and since that time 7 similar cases have been described. It is the purpose of this communication to report the twelfth and thirteenth cases of hemangioblastoma of the spinal cord accompanying Lindau–von Hippel disease. In these cases there was shown, in addition, syringomyelia, and they are the eleventh and twelfth cases to present this combination of lesions.

Fuchs [2] (1882) was the first to describe angiomatosis of the retinas, and Collins [3] (1894) was the first to describe the retinal lesions microscopically. Von Hippel [4] (1895) placed the emphasis on the ophthalmologic signs and symptoms, and since then angiomatosis retinae has been associated with his name. Seidel [5] was the first to note the association of cerebellar and retinal angioblastomas although it was not until after Lindau's article in 1926 that the association became widely known. Cushing and Bailey [6] gave the tumors further prominence and distinguished them histologically from various other vascular tumors.

All types of vascular neoplasms of the spinal cord are uncommon. In a series of 557 intraspinal tumors reported by Rasmussen, Kernohan and Adson,[7] only 64 intramedullary neoplasms were described and but 5 were of vascular origin.

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1. Lindau, A.: Acta path. et microbiol. Scandinav., 1926, supp. 1.

2. Fuchs, E.: Arch. f. Augenh. **11**:440, 1882.

3. Collins, E. T.: Tr. Ophth. Soc. U. Kingdom **14**:141, 1894.

4. von Hippel, E.: Ber. ü. d. Versamml. d. ophth. Gesellsch. **24**:269, 1895.

5. Seidel: Ber. ü. d. Versamml. d. ophth. Gesellsch. **38**:335, 1912.

6. Cushing, H., and Bailey, P.: Tumors Arising from the Blood-Vessels of the Brain, Springfield, Ill., Charles C Thomas, Publisher, 1928.

7. Rasmussen, T. B.; Kernohan, J. W., and Adson, A. W.: Ann. Surg. **111**:513, 1940.

Wolf and Wilens [8] in a review of the literature found only 11 authentic cases of hemangioblastoma of the spinal cord, and added 1 case. They stated that in 5 cases, including their own, there was associated Lindau's disease. In their case syringomyelia and syringobulbia were also shown, and they expressed the belief that it was the fourth case reported in which cystic spinal cord changes occurred with hemangioblastoma and associated Lindau–von Hippel disease.

Hemangioblastoma of the spinal cord occurs frequently in association with Lindau–von Hippel disease, also as an isolated tumor. As has been noted, Lindau [1] found the combination to be present in 4 of 15 cases. Additional cases of hemangioblastoma of the spinal cord associated with Lindau–von Hippel disease have been described.[9] As far as can be determined, the total number of cases of hemangioblastoma of the cord described in the literature (exclusive of the present cases) is 22, in 11 of which it was associated with Lindau–von Hippel disease, and in 10 of these 11 cases syringomyelia was also present. Isolated hemangioblastoma of the cord with syringomyelia has been described in at least 4 cases: Pinner,[10] Tannenberg [11] (case II), Russell,[12] Turner and Kernohan.[13]

REPORT OF CASES

Dr. John Conlon supplied the clinical data in this case.

CASE 1.—A 49 year old white man stated that the onset of his illness occurred in 1919 when, at the age of 29, he noticed, for the first time, that the vision of his left eye was impaired. This condition progressed steadily until by 1935 there was complete loss of vision in the eye. The patient experienced no other symptoms until December 1934, when he began to suffer from sharp jabbing pains in his head on lateral motion. Within a short time he became dizzy when walking, and within a period of two months the dizziness became so pronounced that it was necessary for him to remain in bed. He stated that objects did not revolve but that it was impossible for him to walk straight or to stand. During this period the patient began to suffer from severe occipital and frontal headaches. There was occasional vomiting, unaccompanied by nausea. He was admitted to a hospital, where he was found to have angiomatosis of the right and the left eye. This

8. Wolf, A., and Wilens, S. L.: Am. J. Path. **10**:545, 1934.

9. (*a*) Davison, C.; Brock, S., and Dyke, C. G.: Bull. Neurol. Inst. New York **5**:72, 1936. (*b*) König, E., and Schoen, H.: Beitr. z. klin. Chir. **170**:239, 1939. (*c*) Wolff, K., and Donat, R.: Ztschr. f. urol. Chir. u. Gynäk. **43**:272, 1937. (*d*) Harbitz, F.: Acta path. et microbiol. Scandinav. **11**:442, 1934. (*e*) Knodel, G.: Virchows Arch. f. path. Anat. **281**:886, 1931. (*f*) Craig, W. M.; Wagener, H. P., and Kernohan, J. W.: Arch. Neurol. & Psychiat. **46**:210, 1941. (*g*) Schuback, A.: Ztschr. f. d. ges. Neurol. u. Psychiat. **110**:359, 1927.

10. Pinner, A. W.: Arb. a. d. Geb. d. path. Anat. Inst. zu Tübingen **9**:118, 1914.

11. Tannenberg, J.: Ztschr. f. d. ges. Neurol. u. Psychiat. **92**:119, 1924.

12. Russell, D. S.: J. Path. & Bact. **35**:103, 1932.

13. Turner, O. A., and Kernohan, J. W.: Arch. Neurol. & Psychiat. **46**:444, 1941.

was most marked in the left eye, which was completely blind. The angioma in the right eye was most prominent in the upper nasal quarter of the retina. There was lateral nystagmus, and the reflexes were hyperactive, especially in the left side. There was past pointing to the right with the left hand, as well as clumsiness of the left hand. The patient stood on a wide base, Romberg's sign was positive, and the gait was staggering both to the right and to the left side. A roentgenogram of the skull at this time was essentially normal. A diagnosis of Lindau's disease was made.

On March 20, 1935, cerebellar exploration was performed. The surgeon described the right cerebellar hemisphere as being normal in appearance. In the upper portion of the left cerebellum a cystic area 3 cm. in diameter was found. Associated with this area was a tumor mass which was adherent to the dura in the vicinity of the torcular of the occipital bone. The cyst was opened and all the tumor mass was removed except for a small portion which was adherent to the dura and the torcular. The pathologic diagnosis of the excised tumor was "hemangioma." The postoperative course was uneventful, and the patient was discharged, symptom free, on April 14.

Following the operation, the base of the brain was given 8 exposures to roentgen rays. With the exception of occasional headaches, the patient remained symptom free for four years; then the dizziness suddenly returned together with moderately severe generalized headaches. There was no nausea, vomiting or change in visual efficiency. It was felt that additional surgical intervention was useless, and the patient was admitted to a veteran's hospital for nursing care. The physical examination made at the hospital showed little beyond a weak, emaciated man who had a slightly hyperactive left knee jerk, blindness of the left eye, angioma of the right eye and varicocele of the left side. The patient's condition gradually deteriorated, and he died on July 14, 1940.

During the patient's various hospitalizations, the following significant laboratory data were recorded: The urine was acid, with a specific gravity of 1.022, no albumin and no sugar. Microscopic examination of the urine gave negative results early during his hospitalization and later showed 7 to 8 red blood cells per high power field, no white blood cells and no casts. The Hinton test of the blood was negative. The blood nonprotein nitrogen was 44.4 mg. per hundred cubic centimeters.

Autopsy.—The body was that of a normally developed but poorly nourished white man. The right pupil measured 0.3 cm. and the left 0.2 cm. The left pupil was gray and opaque. There was a posterior median cervical scar 3.5 cm. long and 2.5 cm. wide, also a scar posterior to the left ear 3.5 cm. in length. The heart was normal. There were areas of consolidation throughout both lungs. The pancreas weighed 370 Gm. and measured 29 cm. in length, 9 cm. in width and 4 to 6 cm. in thickness. The organ appeared to be made up almost entirely of cysts measuring from 0.1 to 3 cm. in diameter, with occasional areas of normal-appearing pancreatic tissue between the cysts. The walls of the cysts were paper thin, gray, translucent, and the cysts contained clear straw-colored fluid. The left kidney weighed 490 Gm. and the right 180 Gm. The capsular surface of the left kidney was distorted along the middle and lower portions by a large nodular mass of red and yellow tissue which measured 10 by 5 by 6 cm. When the kidney was sectioned, it was found that the mass had replaced the entire lower two thirds of the kidney and that it extended into and distorted the pelvis of the kidney. The mass itself was made up of golden yellow soft tissue and criss-crossed by streaks of firm white fibrous tissue. Throughout the mass were areas of softening and hemorrhage. At the upper pole of the same kidney was a smaller

and discrete, but otherwise similar, mass which measured 1.0 cm. in diameter. The remainder of the renal parenchyma showed the usual renal structure. Throughout the right kidney were several similar tumor nodules, which varied from 0.5 to 1.5 cm. in diameter. The left pampiniform plexus was markedly dilated. The remainder of the organs other than the brain showed no gross lesions.

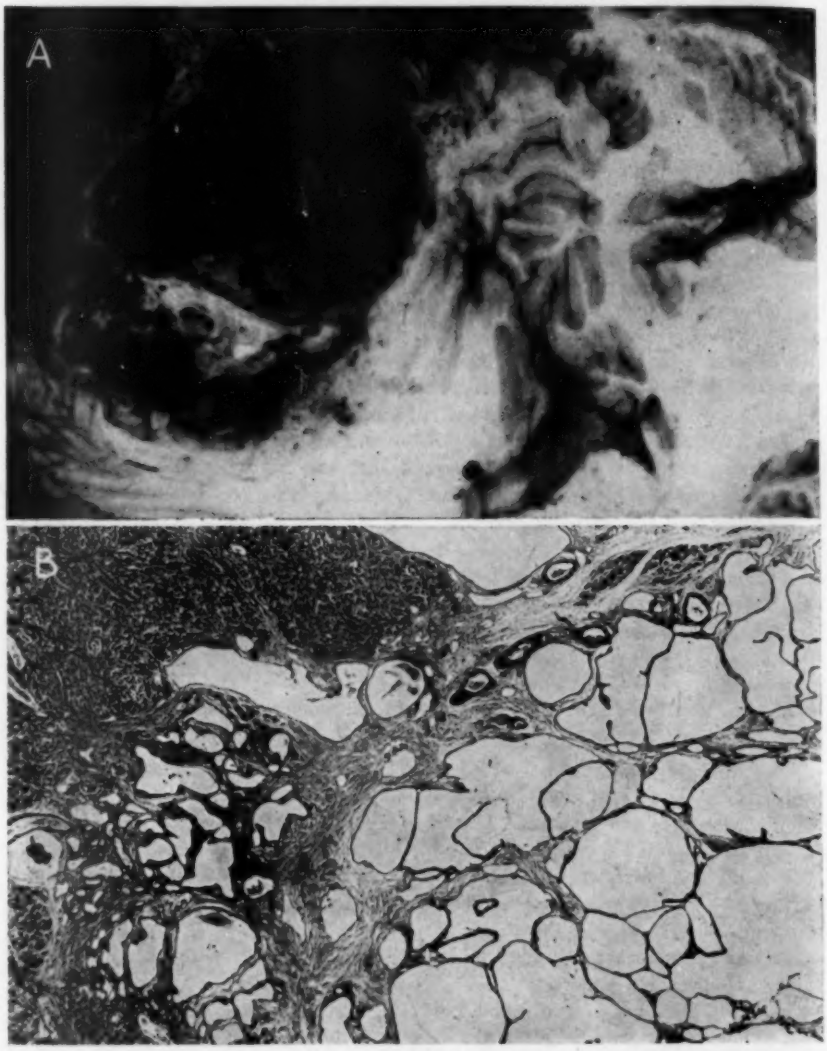

Fig. 1 (case 1).—*A*, left hemisphere of the cerebellum, showing a tumor with cystic spaces containing blood. *B*, section of the cerebellar tumor showing the variation in size of the cysts and some highly cellular areas. Phosphotungstic acid-hematoxylin stain; × 15.

The brain weighed 1,575 Gm. There was moderate flattening of the convolutions of the cerebrum, and the sulci were narrowed. Coronal sections of the

cerebrum did not reveal any abnormalities. There was a dark red cystic mass in the posterior part of the left cerebellar hemisphere, measuring 5.5 by 5.0 by 3 cm. It was made up for the most part of irregular cavities, 1 to 2.5 cm. in diameter. These cavities were smooth-walled and filled with blood. The remainder of the mass was made up of similar but smaller cavities measuring 0.1 to 0.5 cm. (fig. 1 *A*).

The spinal cord showed no gross changes except at the level of about the third to fourth cervical segments. Here one posterior horn was more conspicuous and larger than usual, and the adjacent gray matter was gray-purple. No distinct cavity was seen.

Microscopic Examination.—In the lungs near the larger bronchi were a few small cysts, the walls of which were made up of fibrous tissue covered by a layer of low cuboidal epithelium. There was moderate congestion of the alveolar capillaries, and there were focal areas in which the alveolar spaces were filled by polymorphonuclear leukocytes. The pancreas contained innumerable cysts, varying in size, the walls of which were composed of fibrous tissue lined by low columnar epithelium. In the areas in which these cysts were most numerous, there was considerable fibrosis, with partial replacement of pancreatic acini and compression of neighboring acini. In the regions in which cyst formation was less marked, the usual pancreatic structure was present. The islets of Langerhans did not appear to be involved.

Sections from the tumor masses in the kidneys presented similar histologic pictures. The tumor cells were large and round, with abundant clear cytoplasm and small dark basophilic nuclei. The blood vessels were large and numerous. Considerable hemorrhage and necrosis occurred throughout the tumor. The neoplasm was invasive, and there was no evidence of encapsulation. Sections from the uninvolved renal parenchyma showed no changes.

The retina of the left eye was completely destroyed; the usual layers could not be identified, as they were replaced by an almost continuous band of bone in which there was a small amount of marrow with a few stem cells and nucleated red cells. There were two relatively large tumor masses of thin-walled vessels, and in the intervascular tissue were mononuclear cells containing fat and brown pigment granules. In the choroid there was an increase in the number of chromatophore cells. There was atrophy of the left optic nerve with loss of myelin, extensive gliosis and an increase of connective tissue in the surrounding meninges and perivascular tissue. There appeared to be a decrease in the nerve cells of the retina of the right eye. No tumor was present in the section obtained from the right eye.

The cerebellar tumor was made up of many thin-walled blood vessels lined by endothelial cells. These blood vessels varied in size from capillaries to cavernous vessels, grossly visible (fig. 1 *B*), and red blood cells were present in some lumens (fig. 2 *A*). There were many mononuclear phagocytic cells containing lipid substance in the intravascular tissue. Many poorly formed minute vascular channels, devoid of blood, were lined by endothelial cells. In some places the cells of the intervascular tissue were abundant, mononuclear and possessed of considerable cytoplasm (fig. 2 *A*). There were many large cavities, the walls of which were lined by endothelial cells and which contained no erythrocytes. Reticulum stains showed the capillaries and large vessels to be well outlined and the intervascular tissue to contain much reticulum (fig. 2 *B*). No normal cerebellar tissue was found in the tumor mass.

In the cord, at the level of the third to fourth cervical segments there was an irregularly shaped cavity extending from the posterior commissure into the dorsal

horn laterally. No definite tumor cells were seen in relation to this cavity. In the wall of the cavity were many macrophages, and there was some increase of astrocytes in adjacent tissue. In the cord, at the level of the fourth to fifth

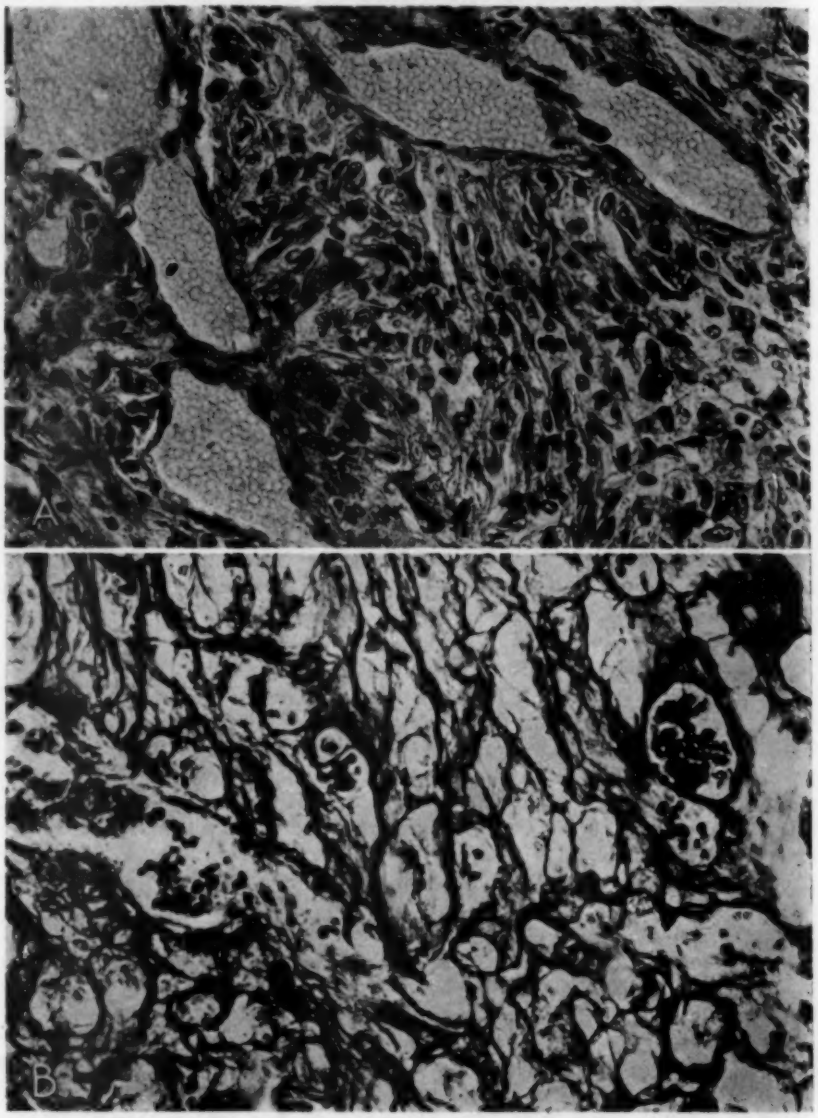

Fig. 2 (case 1).—*A* shows the blood-filled spaces of the cerebellar tumor and the mononuclear cells in the intervascular areas. Phosphotungstic acid–hematoxylin stain; × 400. *B* shows the abundance of reticulum in the vessel walls and in the intervascular tissue of the cerebellar tumor. Perdrau silver stain; × 500.

cervical segments there was tumor in the meninges, in the columns of Clarke and Burdach and in the dorsolateral portion of the cord extending from the roots' entry to within 2 to 3 mm. of the midline and occupying almost all of the afore-

mentioned columns. Adjacent to the tumor were cavities showing no lining cells but surrounded by large astrocytes. The tumor showed the usual vascular channels lined by endothelium with abundant lipid in the cytoplasm. There was an abundance of mononuclear cells together with a few fibroblasts in the intervascular tissue.

Anatomic Diagnoses.—Hemangioblastoma of the cerebellum, left hemisphere, and the cervical portion of the cord; syringomyelia of the cervical portion of the cord; hemangioblastoma of the left eye, with destruction of the retina and bone formation; cysts of the pancreas; cysts of the lungs; bilateral hypernephroma; bronchopneumonia of the lower lobes of the right and left lungs, and varicocele of the left side.

In case 1 the patient experienced impairment of vision at the age of 29 years, and it was not until sixteen years later that signs of cerebellar tumor appeared. One of the striking features of this case is the completeness of the Lindau–von Hippel complex, as the cerebellum, the retinas, the spinal cord, the lungs, the pancreas and the kidneys were involved. This is one of the few cases recorded in which the visceral lesions produced clinical signs, since the microscopic hematuria and the varicocele of the left side were probably related to the hypernephroma. The lesion of the spinal cord is of considerable interest because of the association of hemangioblastoma of the spinal cord and syringomyelia with the classic Lindau–von Hippel lesions of the cerebellum and the retina. There were no neurologic signs or symptoms which could be attributed to the involvement of the spinal cord.

Case 2.—M. Z., a 27 year old single white woman, was admitted to the Boston City Hospital, Dec. 7, 1936, because she was unable to walk without assistance. The past history is interesting in that at the age of 2 years she underwent surgical immobilization of the upper thoracic region for presumable Pott's disease. When she was 25 years of age, radical mastoidectomy was performed on the left side at the Boston City Hospital for chronic discharge of the ear, and a polyp was removed from the posterior wall of the external meatus. The patient stated at that time that she occasionally suffered from dizziness but that she never fell. She also complained of partial blindness of the left eye, and she had vision in only the lower visual field. Examination of the left fundus showed old retinitis proliferans with detached retina. The right eye was normal. The history of her family was not remarkable.

The patient dated the onset of her present illness to June 1933, three and a half years before the final admission; at that time she began to suffer from hiccup, which usually came on at night and which was occasionally accompanied with nausea and vomiting. She stated that she had always been deaf in the left ear. Shortly after the mastoidectomy, in 1935, she began to suffer from headaches. Two months before admission, in 1936, she had daily headaches which were almost continuous. One year before admission she became completely blind in the left eye. Four weeks before admission she noticed weakening of both legs and numbness of the toes. This appeared to be more marked on the right than on the left side.

On admission her temperature was 98.4 F., the pulse rate 92 and the respiratory rate 22 per minute, and the blood pressure 90 mm. of mercury systolic and 70 diastolic. The patient was a poorly nourished and poorly developed woman in

no great discomfort. There was marked sharp flexion deformity in the region of the first and second dorsal vertebrae, and there was an old healed operative scar over this deformity. The spinal column was otherwise not remarkable. There was a discharge of the left ear, and a mastoidectomy scar was present on the left side. The left eye was blind, and there was complete separation of the retina with evidence of old retinal hemorrhage. The right optic disk showed choking of 4 diopters, with small hemorrhages visible about the optic disk. There was a large beaded vessel, apparently an artery, extending laterally and inferiorly from the disk. This vessel disappeared at the margin of a circular white area located at about 8 o'clock, which was slightly larger than the optic disk and was surrounded by a broken narrow rim of black pigment. Two large vessels were seen at the lateral side of the area, but their course could not be followed farther. The cranial nerves were otherwise not abnormal. The upper extremities were not remarkable, but there was marked weakness of the lower extremities, more marked on the right. There were poorly sustained left ankle clonus and bilateral ataxia. The Kernig signs were not present. There was no definite level of sensory loss, but there seemed to be anesthesia to pain and touch in about the distribution of the fifth lumbar nerve bilaterally, and there was markedly diminished vibration sense at both ankles and absence of position sense in the legs. Both biceps reflexes and the left radial reflex were hyperactive. The abdominal reflex of the lower left quadrant was absent. The right knee jerk was diminished and the left increased. The left ankle jerk was more active than the right. There were positive bilateral Babinski responses. A diagnosis of Lindau's disease was made, and a cerebellar exploration was carried out. At operation a cystic tumor was found in the medulla and another in the cord. It was thought to be unwise to attempt removal of either of these tumors. Following operation, the patient's temperature was 102 F.; the pulse rate was 120 to 180. She complained of headache, later became unresponsive, and died two days after operation.

On admission the hemoglobin was 80 per cent (Sahli); the red blood cell count was 4,100,000 and the white blood cell count 6,000 per cubic millimeter. The examination of the urine gave negative results. The Hinton test of the blood was negative. Lumbar punctures on three occasions showed initial pressure between 90 and 100 mm., with no rise on jugular compression, but a rise of 200 mm. on abdominal compression. A cisternal puncture showed an initial pressure of 350 mm., with a rise to 500 mm. and a rise to 450 mm. on jugular and abdominal compression, respectively.

The specimens of cerebrospinal fluid obtained from lumbar punctures and from the ventricular puncture made at the time of operation were clear, slightly yellow and without clot. They showed no white blood cells, but on some occasions a few red blood cells. Fluid obtained by lumbar puncture gave a 4 plus reaction in the Pandy test and contained between 420 and 600 mg. of protein per hundred cubic centimeters. The Lange colloidal gold curve was 1222333432, and the Wassermann test was negative. Fluid obtained by cisternal puncture gave a 1 plus reaction in the Pandy test and contained 150 mg. of protein per hundred cubic centimeters.

Roentgenograms of the mastoid processes showed sclerosis and destruction of bone on the left side. A roentgenogram of the skull showed no abnormality. A roentgen examination of the chest revealed normal lung fields, enlarged hilar glands and marked distortion of the spinal column.

Postmortem Examination.—The body was that of a fairly well nourished white woman showing generally immature development. There was a recent

operative scar in the occipital region extending 21 cm. from side to side and 8 cm. caudal from the midpoint of the lateral incision. There was marked kyphosis of the upper dorsal portion of the spinal column. With the exception of the central nervous system, there were no gross lesions demonstrable in any of the organs of the body. There was an oval operative bony defect 8 by 5 cm. in the occipital region. The exposed sutured dura was covered by a thin seropurulent exudate. The brain weighed 1,370 Gm. There was considerable flattening of the convolutions with narrowing of the sulci. The meninges at the base of the brain and over the posterior portions of the temporal lobes were covered by thin yellow purulent exudate. This exudate was also present above the tentorium at the junction of the sagittal and transverse fissures. When frontal sections were cut, bilateral hydrocephalus was demonstrated, and each of the lateral ventricles measured 2.7 by 2 cm. Beneath the ependyma of both the lateral and third ventricles were multiple petechial hemorrhages averaging 0.1 to 0.2 cm. in diameter. The cerebellum itself was normal, but the posterior portion of the fourth ventricle, the foramen of Magendie and the posterior portion of the cisterna magna were involved by a neoplastic mass measuring 1.7 by 1.5 by 1 cm. This mass apparently arose from the floor of the fourth ventricle and there was only slight involvement of the cerebellum. It was firmer in consistence than the surrounding brain tissue, and its color was pink-gray. Two small cystic channels appeared ventrally near the attachment of the tumor to the floor of the fourth ventricle; one measured 0.4 by 0.3 cm. and the other 0.4 by 0.8 cm. The pituitary gland was covered by thick purulent fluid.

The arches of the first and second cervical vertebrae had been removed. There was ankylosis of the first six dorsal vertebrae with marked kyphosis, and the peak of angulation was at the level of the fifth dorsal vertebra. The cord correspondingly showed marked angulation at this level, and it was narrowed here so that it measured 1 by 0.65 cm. in cross section.

The entire cervical portion of the cord was swollen, and this was most marked in the lower five segments. The upper four segments were fluctuant. Multiple sections of the cord showed the tumor of the fourth ventricle extending caudad through the left nucleus of Burdach and the left substantia gelatinosa Rolandi into the left posterior horn and left posterior column of the cervical and dorsal segments of the spinal cord. The tumor extended downward to the level of the eleventh dorsal segment. It contained a continuous cystic cavity extending from the first cervical to the sixth dorsal segment and a small cyst, 0.1 cm. in diameter, in the left posterior column at the eleventh dorsal segment. In addition, there was a small and apparently independent tumor nodule, measuring 0.2 cm. in diameter, in the left lateral column at the level of the fifth cervical segment.

The posterior third of each orbit was removed. On the left the choroid was detached with the exception of a small area laterally. The remainder lay in the posterior chamber and was adherent to the posterior surface of the lens. In the right eye there was a small pink elevation, 2 cm. in diameter, at the extreme right of the choroid.

Microscopic Examination.—In the medulla there was a tumor nodule arising from the posterior medullary velum and protruding into the fourth ventricle. The nodule was composed of many capillaries, as well as larger vessels of varying size lined with endothelium and containing red blood cells. Between the vessels was a fairly heavy stroma varying from isolated fibroblasts to many mononuclear cells, some containing lipid. The tumor in the cerebellum was similar to that seen in case 1. The nodule extended into the lateral portion of the fourth ventricle but it

was impossible to determine from the material available whether the area postrema was involved. Ventral and lateral to the tumor nodule was a large cavity, the walls of which were formed by proliferated astrocytes, Along one margin of the cavity were a large focus of polymorphonuclear and mononuclear cells and a few

Fig. 3 (case 2).—Spinal cord at the level of the eighth cervical segment, showing tumor and a syrinx. Hematoxylin and eosin stain; × 15.

adjacent foci of capillaries filled with red blood cells which were surrounded by a zone of histiocytes. The cavity occupied the dorsal lateral portion of the medulla and extended into the restiform body on one side. The ependyma around the

fourth ventricle showed several petechial hemorrhages, and there was fibrinopurulent exudate in the substance of the medulla, particularly around the larger vessels, and the ependyma.

Fig. 4 (case 2).—Spinal cord at the level of the first dorsal segment, showing a syrinx with a wall of glial tissue and absence of tumor. Hematoxylin and eosin stain; × 15.

A small nodule of tumor tissue similar to that described in the foregoing paragraph was found in the stalk of the pituitary gland.

Histologic sections revealed tumor in the left posterior horn and the left half of the posterior column from the first cervical to the eleventh dorsal segment (fig. 2 *B*) and a small, apparently independent nodule, measuring 0.2 by 0.175 cm., in the left lateral column at the fifth cervical segment. The tumor tissue of the spinal cord was identical to that of the floor of the fourth ventricle and impinged on a cystic cavity which extended from the first cervical to the sixth dorsal segment (fig. 3). There were small areas of cavitation in the neighborhood of the tumor in the left posterior horn at the fourth and fifth cervical segments, in both anterior horns at the seventh cervical segment, in both posterior horns at the eighth cervical segment, in both anterior horns and the right posterior horn at the first and third dorsal segments. The walls of the cavity showed hyperplasia of large glial astrocytes, and no tumor cells were present (fig. 4). Areas of necrosis were seen in the gray matter of the left side from the eighth to the tenth dorsal segments and in both anterior horns at the eleventh dorsal segment, and there was marked gliosis in the posterior columns from the eighth to the eleventh dorsal segment, corresponding to the angulation of the gibbus of the spinal column.

There was polymorphonuclear infiltration of the meninges of the cord extending from the medulla oblongata downward to the second lumbar segment. This infiltration was massive down to the level of the fifth dorsal segment and only slight from the fifth dorsal to the second lumbar segment. The choroid plexus was covered with purulent exudate.

Permission was obtained to remove only the posterior portion of each retina. The retina of the left eye showed detachment with folding, as well as hyperplasia of large chromatophores and prominent capillaries, but there was no definite tumor formation. The retina of the right eye showed no tumor and no detachment. It is possible that the vascular and capillary hyperplasia seen in the left retina represented tissue taken from the edge of a tumor similar to that of the medulla oblongata and the cord.

Several small cysts were present in the lungs, and the majority of these were located in small clusters at the periphery of the lung. The walls of the cysts were made up of fibrous tissue and were lined by low columnar epithelium. There were foci of alveoli filled with polymorphonuclear cells, bacteria and debris.

There were a few small cystic spaces in the pancreas, which were lined by low columnar epithelium showing clear cytoplasm and large nuclei with diffusely scattered chromatin. The cysts appeared to cause no distortion of the usual pancreatic structure.

Sections taken from the vertebral column at the point of acute angulation showed destruction of bone and replacement by fibrous tissue. There was no evidence of tumor or inflammation.

Anatomic Diagnoses.—Hemangioblastoma involving the floor of the fourth ventricle and the spinal cord (first cervical to eleventh dorsal segment); syringomyelia (first cervical to sixth dorsal segment and at the eleventh dorsal segment); syringobulbia; cysts of the pancreas; cysts of the lungs; purulent meningitis; marked kyphosis with ankylosis of the first to the sixth dorsal vertebrae; healed fibrous pleuritis of the upper and middle lobes of the right lung; bronchopneumonia of the lower lobe of the left lung.

The clinical history in case 2 is similar to that in case 1 in that the first symptom was impairment of vision followed later by signs and symptoms referable to a disturbance of the central nervous system.

The marked kyphosis was a confusing factor in this case. It was first thought that there was compression of the cord from Pott's disease and that there were associated tuberculomas of the cerebellum and the eye. However, the diagnosis of Lindau's disease was made and subsequently confirmed by cerebellar exploration.

As far as can be determined, this is the twelfth case to be reported in which syringomyelia was associated with hemangioblastoma of the spinal cord and the thirteenth case in which hemangioblastoma of the cord was associated with the Lindau–von Hippel syndrome. Syringobulbia was also present.

COMMENT

Lindau–von Hippel disease is uncommon. Symptoms usually appear between the second and fourth decades, and the disease shows no particular sex incidence. Lindau [14] stated that 25 per cent of the patients suffering from von Hippel disease will show symptoms due to associated cerebellar lesions. He pointed out that a familial factor can be demonstrated in approximately 20 per cent of all cases of this syndrome. Thomsen,[15] from his studies, expressed the belief that the disease is transmitted by a single dominant factor, not sex linked. Möller [16] was able to demonstrate 6 single or combined retinal and cerebellar tumors in three generations, and on the basis of the pronounced hereditary factor he was able to diagnose correctly, for the first time postoperatively, an intracranial hemangioblastoma. With a family history of the disease or the demonstration of retinal angioma and with the presence of signs of disturbance of the cerebellum or of the fourth ventricle, a probable diagnosis of Lindau–von Hippel disease can be made.

Numerous investigators have postulated a congenital origin for hemangioblastoma. The work of Sabin [17] and Karlefors [18] has shown that in the third fetal month a vascular mesenchyme is situated in or adjacent to the posterior end of the fourth ventricle (velum medullare posterior), and Lindau expressed the belief that since the cerebellar hemispheres develop at about this period a part of the original vascular mesenchyme might be drawn into the cerebellar hemispheres. Vascularization of the retina, ingrowth of the mesodermal elements and the branching anlage of the pancreas also take place in the third fetal

14. Lindau, A.; Sargent, P., and Collins, E. T.: Proc. Roy. Soc. Med. **24**:363, 1931.

15. Thomsen, O., cited by Möller.[16]

16. Möller, H. V.: Acta ophth. **7**:244, 1929.

17. Sabin, F. R.: Anat. Rec. **13**:199, 1917.

18. Karlefors, J.: Die Hirnhauträume des Kleinhirns, Stockholm, Norstedt & Söner, 1924.

month, and it is possible that the other associated developmental defects occasionally seen in patients with Lindau–von Hippel disease occur at this period.

Cushing and Bailey [6] divided vascular tumors of the central nervous system into two groups: angiomas and angioblastomas, a subdivision which has been generally recognized. The angiomas were considered to be vascular malformations consisting of fully formed blood vessels with an intervening stroma of normal nerve tissue. Angioblastomas were considered to be true tumors capable of slow progressive growth, which rarely show mitoses or giant cell formation and which do not metastasize or spread along the ventricular or subarachnoid spaces. These tumors are composed of an extensive new growth of vascular channels containing red blood cells and lined with endothelial cells. Reticulum stains show a characteristic pattern of outlined capillaries and intercapillary bridges. Considerable variation is seen in the amount of intervascular tissue; some tumors show capillary arrangement with little or no intervening tissue, while others reveal marked intervascular cellularity with many minute, poorly formed vascular spaces, usually empty, and lined by either well formed adult endothelial cells or by more primitive cells—the latter being presumably immature endothelial cells. The tumor cells typically have an oval vesicular nucleus and pale cytoplasm containing a few vacuoles. Nucleoli are not uncommon. Mitoses are rare. Occasionally the lipoid content of the interstitial substance is marked and overshadows the vascular picture. This lipoid material is contained in large mononuclear phagocytic cells. Lindau expressed the belief that the vascular tumor with its large blood-filled spaces caused pressure necrosis of the nerve tissue with consequent liberation of lipoid, which was subsequently phagocytosed by the endothelial elements of the tumor. The tumors frequently show areas of regressive changes, such as edema, hyalinization, calcification and occasionally fatty degeneration.

A further differentiation of the types of hemangioblastoma was made by Cushing and Bailey [6] on the basis of structure. The type showing predominence of narrow vascular channels with thin septums was described as capillary; that showing widely dilated channels, as cavernous, and a type showing a relative paucity of vessels and predominance of cellular elements was designated as celluiar. This further subdivision of hemangioblastomas does not appear to be valid, as all types of hemangioblastoma are frequently seen in different sections of the same tumor, although one type may predominate.

Cerebellar hemangioblastomas in general comprise less than 1 per cent of intracranial tumors. In patients with Lindau–von Hippel disease their presence usually gives rise to cerebellar symptoms some years

after the ocular changes. The average age at onset of the cerebral lesions is 32 years (Lindau). These tumors may be accompanied by hemorrhage and edema. Lindau collected from the literature and his own material 40 cases of cystic hemangioblastic tumor of the cerebellum, and he gives the impression that in most instances the tumor is cystic although in a few it is solid or only partially cystic. The neoplasm manifests itself by the usual signs and symptoms of a cerebellar tumor, and there is nothing distinctive that allows it to be clinically differentiated from other cerebellar tumors. It has been emphasized by Dandy [19] and Cushing [6] that in the cystic type the small tumor nodule usually found on the cyst wall must be removed to prevent refilling of the cavity, as aspiration of the cyst is not sufficient for cure. The tumor in case 1 was partially cystic.

Hemangioblastoma may rarely occur in other portions of the rhombencephalon as in case 2. This is the finding in 2 of the previously reported cases of Lindau–von Hippel disease with involvement of the cord (Knodel [9e] and Schuback [9g]).

When a lesion of the spinal cord is present with Lindau–von Hippel disease, it is usually a hemangioblastoma. It lies in the dorsal half of the cord, usually in the posterior columns and adjacent to the posterior septum. The tumor has occurred at all levels of the cord but shows a predilection for the cervical and lumbar regions. It has been observed in the cauda equina and in nerve roots. The fact that the Lindau–von Hippel complex is present in approximately half of the reported cases of hemangioblastoma of the cord suggests the possibility that closer examination of the spinal cord in cases of Lindau–von Hippel disease would reveal an even higher incidence of hemangioblastoma of the cord.

The syringomyelic cavities associated with hemangioblastoma of the spinal cord are surrounded by a dense glial wall, and they are not lined by ependymal cells. Davison, Brock and Dyke [9a] expressed the belief that the cavities are caused by the tumor's destroying or softening of the cord or by its pressing on the intraspinal arteries, with circulatory interference and subsequent cyst formation. Bielschowsky and Unger [20] expressed the belief that the cavity and concomitant vascular lesion were congenital anomalies. Lindau considered the origin of the syringomyelia to be similar to that of the formation of a cerebellar cyst: i. e., transudation of fluid from the vessels of the tumor with formation of cysts and compression of adjacent glial tissue resulting in a wall of compressed glia without an ependyma. There was marked cavity formation throughout the cord and medulla in case 2, and to a lesser

19. Dandy, W. E.: The Brain, in Lewis, D.: Practice of Surgery, Hagerstown, Md., W. F. Prior Company, Inc., 1944, vol. 12, sect. 12, p. 640.

20. Bielschowsky, M., and Unger, E.: J. f. Psychol. u. Neurol. **25**:173, 1920.

degree in the cord in case 1. In both cases the walls of the cavities were composed of dense glial tissue, and there was no evidence of an ependymal lining. It is of interest to note that distinct areas of typical syringomyelia were found in which there were no associated vascular lesions.

The ocular lesion is a hemangioblastoma of the retina similar to the vascular tumors found elsewhere. Symptoms from the retinal tumor generally appear at about the age of 25 years, although occasionally the onset is in childhood, but rarely does it occur after the age of 45 years.[21] It is stated that retinal angiomatosis is twice as frequent in males as in females.[8] At first, usually only slight impairment of vision of one eye is noted. Later the other eye may show involvement, or it may remain free from the disease. The ophthalmoscopic picture (Craig, Wagener and Kernohan [9f]) in the early stage is that of a round red angiomatous mass in the periphery of the retina, and issuing from the mass are two large dilated vessels, artery and vein, running parallel to each other and to the optic disk. Occasionally diffuse dilatation of all retinal vessels without the angiomatous mass is present.

The retinal lesion is progressive, though its rate of growth is variable, and the vision gradually lessens as the retinal and subretinal edema from the tumor gives rise to local and generalized detachment of the retina, hemorrhage, gliosis, secondary glaucoma and blindness. The detachment of the retina and the reactive gliosis and connective tissue proliferation lead to a puzzling secondary ophthalmologic picture that obscures the primary process and is most commonly mistaken for Coats's "exudative retinitis" (Lindau). Calcification and rarely ossification may supervene and leave a monumental stigma visible on roentgen examination of the orbit (Davison, Brock and Dyke [9a]). In case 1 there were hemangioblastoma, calcification, and ossification with myelopoiesis of the left retina; in case 2 there was detachment of the left retina, and although the diagnosis was made clinically, no tumor was demonstrated conclusively at autopsy, as only a portion of each eye could be removed.

The accompanying visceral lesions are rarely vascular in origin, and most frequently they take the form of cysts of the pancreas and the kidney. The kidney, and rarely the adrenal gland, may show "hypernephromata." In 6 of Lindau's [1] original 15 cases there were "hypernephroid" tumors of the kidneys and others have been described since.[22] Cahill, Melicow and Guerry [23] have described a case in which the renal

21. Brandt, R.: Arch. f. Ophth. **106**:127, 1921.

22. Wolf and Wilens.[8] Davison and others.[9a] König and Schoen.[9b] Craig and others.[9f]

23. Cahill, G. F.; Melicow, M. M., and Guerry, Du P., III: Tr. Am. A. Genito-Urin. Surgeons (1942) **35**:271, 1943.

tumor was a hemangioblastoma. Less commonly tumors of the epididymis and angioma of the liver are found.

In general, the visceral lesions cause no symptoms, but the patient of Davison, Brock and Dyke [9a] had abdominal complaints, and at operation a polycystic tumor of the lesser peritoneal sac attached to the pancreas was demonstrated. Microscopic examination revealed a cystadenoma of the pancreas. In case 1 the bilateral hypernephromas were associated with microscopic hematuria and with varicocele of the left side.

SUMMARY

Two cases of Lindau–von Hippel disease with hemangioblastoma of the spinal cord and syringomyelia are presented. The literature is reviewed, and the nature and the genesis of the lesions are discussed.

ROLE OF LYMPHATIC VESSELS IN THE TRANSMISSION OF LIPASE IN DISSEMINATED PANCREATIC FAT NECROSIS

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SO-CALLED pancreatic and peripancreatic fat necrosis is supposedly due to the splitting of neutral fat into glycerol and free fatty acids by pancreatic lipase which has escaped from the injured pancreas. The free fatty acids are thought to combine subsequently with calcium in the tissue and tissue fluids to form insoluble calcium soaps, and it is these products which give rise to the opaque white areas in the fat depots of the abdominal cavity and elsewhere.

Fat necrosis may follow many types of injury of the pancreas, such as acute pancreatitis, primary and metastatic carcinoma of the pancreas, obstruction of the pancreatic ducts and trauma. Indeed, Opie [1] pointed out that fat necrosis bears somewhat the same relationship to pancreatic disease that jaundice does to diseases of the liver.

The mode of transmision of lipase from the pancreas to the sites of fat necrosis has given rise to much speculation, and a review of the literature revealed that the question is still unsettled.

The purpose of the studies reported in this paper was to determine the role of the lymphatic vessels in fat necrosis and to endeavor to demonstrate that these channnels are the primary means of transmission of escaped pancreatic lipase.

REVIEW OF THE LITERATURE

Pancreatic and peripancreatic fat necrosis has been mentioned by a number of the early writers, including Schmidt [2] (1818), Virchow [3]

From the Mayo Foundation.

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1. Opie, E. L.: Diseases of the Pancreas, ed. 2, Philadelphia, J. B. Lippincott Company, 1910, pp. 176-199.

2. Schmidt, cited by Flexner, S.: Johns Hopkins Hosp. Rep. **9**:743, 1900.

3. Virchow, R., cited by Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1929, vol. 5, pt. 2, pp. 358-371.

(1852) and Ponfick [4] (1871), but Balser [5] gave his classic gross and microscopic description of what he termed *Fettenekrosen* in 1882. He thought the necrosis occurred because overgrowth of fat tissue cut off the blood supply.

Fitz [6] in 1889 first pointed out the intimate relationship of fat necrosis to disorders of the pancreas. He described a number of cases of acute pancreatitis associated with fat necrosis.

In 1890 Langerhans [7] substantiated Fitz's ideas by producing fat necrosis experimentally. He did this by injecting extracts of rabbit pancreas intraperitoneally in dogs. Langerhans also showed by chemical means that fatty acids and calcium were both present in the necrotic patches, probably in combination as calcium soaps.

In 1897 Flexner [8] showed in an experimental study that the small regions in which fat necrosis had occurred in animals had a much higher content of lipolytic substance than the surrounding fat.

Wells [9] in 1903 produced experimental fat necrosis in dogs and cats by intraperitoneal injection of extracts of hog pancreas and also of solutions of commercial pancreatin. Both grossly and microscopically these necrotic patches were exactly like those in human beings. Wells found that if his extracts and solutions were heated to about 71 C., all lipolytic activity was lost, and so it was thought that the active substance was an enzyme. Wells pointed out that, although other workers had failed to produce fat necrosis with solutions of trypsin weak in or devoid of lipolytic substance, it could not be definitely stated that lipase alone was responsible for fat necrosis, because at that time, on account of technical difficulties, it was impossible to separate the two enzymes.

For years it was thought that fat necrosis depended on the action of both trypsin and lipase. The trypsin supposedly killed the fat cells first and then the lipase split the neutral fat. Neal and Ellis,[10] however, showed that lipase alone could cause extensive fat necrosis. They proved this by intraperitoneal injection of extracts of hog pancreas which had no tryptic activity and with lipolytic extracts of dried peanuts and sunflower seeds, using dogs.

The exact nature of the material contained in the regions of fat necrosis is controversial. It has been mentioned before that Langerhans concluded from his chemical and histologic observations that free fatty acids and calcium were both present, probably in combination as calcium soaps. It is generally agreed, however, that it is a difficult problem to

4. Ponfick, E., cited by Neal, M. P., and Ellis, M. M.: South. M. J. **23**:313, 1930.
5. Balser, W.: Virchows Arch. f. path. Anat. **90**:520, 1882.
6. Fitz, R. H.: M. Rec. **35**:197, 1889.
7. Langerhans, R.: Virchows Arch. f. path. Anat. **122**:252, 1890.
8. Flexner, S.: J. Exper. Med. **2**:413, 1897.
9. Wells, H. G.: J. M. Research **9**:70, 1903.
10. Neal, M. P., and Ellis, M. M.: South. M. J. **23**:313, 1930.

distinguish between the various types of fat and lipoid crystals chemically or histologically.

According to Herbert,[11] the necrotic patches contain neutral fat, fatty acids and calcium soaps. Her tests showed that relatively small amounts of the fatty acids were in actual combination with calcium in spite of the fact that these patches contain more calcium than the surrounding fat.

Opie produced fat necrosis in cats by ligating both pancreatic ducts. He not only produced necrosis in the usual sites of abdominal fat by this technic but also found evidence of disseminated fat necrosis in the pericardial, subpleural and subcutaneous fat. According to Opie, clinical cases of fat necrosis associated with pancreatic disease in which similar observations were made have been reported in the literature, and he felt that pancreatic lipase was carried to the sites of necrosis in both the abdominal and the thoracic cavity by the lymphatics.

Rostock,[12] a German worker, reviewed the literature on the transmission of lipase in fat necrosis and pointed out that the earlier workers courted three principal hypotheses. They thought that transmission of lipase occurred by (1) direct contact, (2) blood stream transport of either lipase or pancreatic acinous cell emboli and (3) lymphatic transport. Rostock concluded from his studies that most of the evidence favored the hypothesis of transmission by way of the lymphatics.

In order to prove this point more conclusively, he carried out an interesting study in which he used material taken from necrotic regions which had been produced in dogs. When these pieces of tissue were placed in strong hydrogen peroxide, the lymph channels were delineated because oxygen bubbles were liberated as the result of the catalytic action of lymph. Rostock was able to demonstrate microscopically that lymph vessels were present in the tissue adjacent to the sites in which fat necrosis had occurred.

PRESENT STUDY [13]

It occurred to me that if the hypothesis concerning the lymph channels was correct, a close association between the lymphatic vessels and sites of fat necrosis would be shown if a suitable lipolytic material were mixed with a lymphatic-delineating substance and injected intraperitoneally in rats. If, on the other hand, it was not true, no such association would be shown.

Wells and other workers had used solutions of commercial pancreatin to produce fat necrosis in animals, and this seemed the best lipolytic material for my purpose.

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11. Herbert, F. K.: Brit. J. Exper. Path. **9**:57, 1928.

12. Rostock, P.: Beitr. z. klin. Chir. **138**:171, 1926.

13. The work was done in the Section on Experimental Bacteriology of the Mayo Foundation under the direction of Dr. H. E. Robertson.

For the delineation of the lymph channels it was decided to use a suspension of finely particulate graphite called "hydrokollag 300." [14]

Preliminary Studies.—Several large normal white rats were killed with ether and necropsy was performed in order to review the normal anatomy of the rat.

In the second group of rats an intraperitoneal injection of a 4 per cent solution of pancreatin [15] in isotonic solution of sodium chloride was given. After twenty-four hours the animals were killed, and examination revealed many small necrotic regions throughout the fat depots of the peritoneal cavity and also in the subpleural and mediastinal fat.

The suspension of finely particulate graphite was injected alone intraperitoneally in other rats to determine whether it had any lipolytic activity. The animals were killed at the end of twenty-four hours, and necropsy was performed. Neither gross nor microscopic examination showed any evidence of fat necrosis. The lymph channels throughout the abdominal cavities and viscera of these rats, however, were delineated as fine black weblike lines. It was also apparent that in the rat there are lymph channels between the peritoneal cavity and the mediastinal lymph nodes similar to those found in the dog by Higgins and Graham.[16] The lymphatics associated with the internal mammary and other mediastinal blood vessels were delineated, and the mediastinal lymph nodes were black with graphite, while the nodes observed in the normal animal were pink.

Major Study.—Twenty-five large white rats were used for this study. An intraperitoneal injection of a mixture of the solution of pancreatin and the graphite suspension was made. Each animal received 6 cc. of the 4 per cent solution of pancreatin in isotonic solution of sodium chloride mixed with 1 cc. of the graphite suspension.

All of the animals survived for at least four hours. Nine of the rats were found dead after eighteen hours, but 16 animals were alive and appeared to be in good condition. Four of the remaining 16 rats were killed eighteen hours, 9 twenty-four hours, 2 twenty-six hours and 1 forty-eight hours after injection.

Gross Examination: The 25 animals were all examined. The peritoneal cavities were opened and found to contain from 3 to 8 cc. of dirty gray fluid. Sites in which evidence of typical fat necrosis was found were numerous and varied in size from pinpoint diameter to 0.5 cm. Evidence of necrosis was found throughout the omental, mesenteric, perirenal and subperitoneal fat. The epididymis of each male rat was also involved. The lymph channels were delineated by the graphite as previously described, and there appeared to be a definite association between these channels and the small regions of fat necrosis. In many instances the fine black lines either terminated directly in the saponified material or surrounded it in a close network. Frequently the saponified material was tinted gray by the infiltration of graphite particles. On gross examination a few lesions were found, especially in the perirenal fat, which were not associated with the delineated channels.

No diaphragmatic apertures between the peritoneal and the thoracic cavity were visible on gross examination.

The thoracic cavity was opened by cutting the ribs along their lateral margins and turning back the anterior portion of the thoracic wall. In no instance did the thoracic cavity contain fluid. The sternal and other mediastinal lymph channels

14. Higgins, G. M., and Murphy, G. T.: Anat. Rec. **40**:15, 1928.
15. Lot no. 3254675, supplied by Parke, Davis & Company, Detroit.
16. Higgins, G. M., and Graham, A. S.: Arch. Surg. **19**:453, 1929.

were delineated, and the mediastinal lymph nodes were black with graphite in all instances. In every rat evidence of fat necrosis was found at some point along these lymphatics. The lesions were found in the fat along the sternal channels, in the adipose tissue lying in front of the inferior venae cavae and in the fat immediately adjacent to the blackened mediastinal nodes (fig. 1 *a*).

The findings in the 9 rats that died and the 16 that were killed with ether were essentially the same.

Histologic Examination: Tissue was taken from each of the 25 animals for microscopic study. Included were sections of diaphragm and patches of fat necrosis from the peritoneal fat, the anterior thoracic wall, the fat about the inferior venae cavae and the mediastinal nodes and adjacent fat.

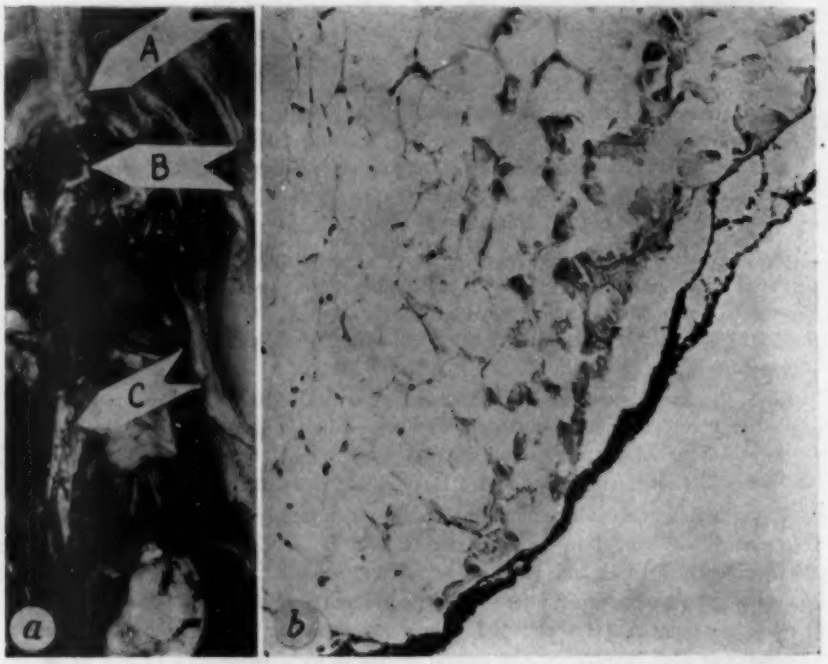

Fig. 1.—(*a*) The marker *A* indicates a left sternal lymph channel delineated by graphite and associated sites of fat necrosis on the posterior surface of the anterior portion of the thoracic wall; *B*, a mediastinal lymph node filled with graphite and surrounded by a zone of fat necrosis; *C*, multiple small necrotic regions in the adipose tissue about the inferior vena cava between the heart and the diaphragm. Unfortunately, the delineated lymphatic vessels were too fine to show up well in this illustration.

(*b*) A site of fat necrosis from the mesentery showing the related graphite-filled lymph channels. Under higher magnification graphite particles could also be seen infiltrating the saponified material.

Examination of many of the small regions of fat necrosis from the various peritoneal sites revealed a constant relationship between these lesions and graphite-filled lymphatics which varied in size from tiny intercellular spaces to channels easily visible macroscopically. Frequently graphite particles were also found infiltrating the saponified material of the lesions (fig. 1 *b*).

Sections of diaphragm were studied, and graphite was found in the lymph channels. The sternal lymphatics of the anterior thoracic wall were also found to

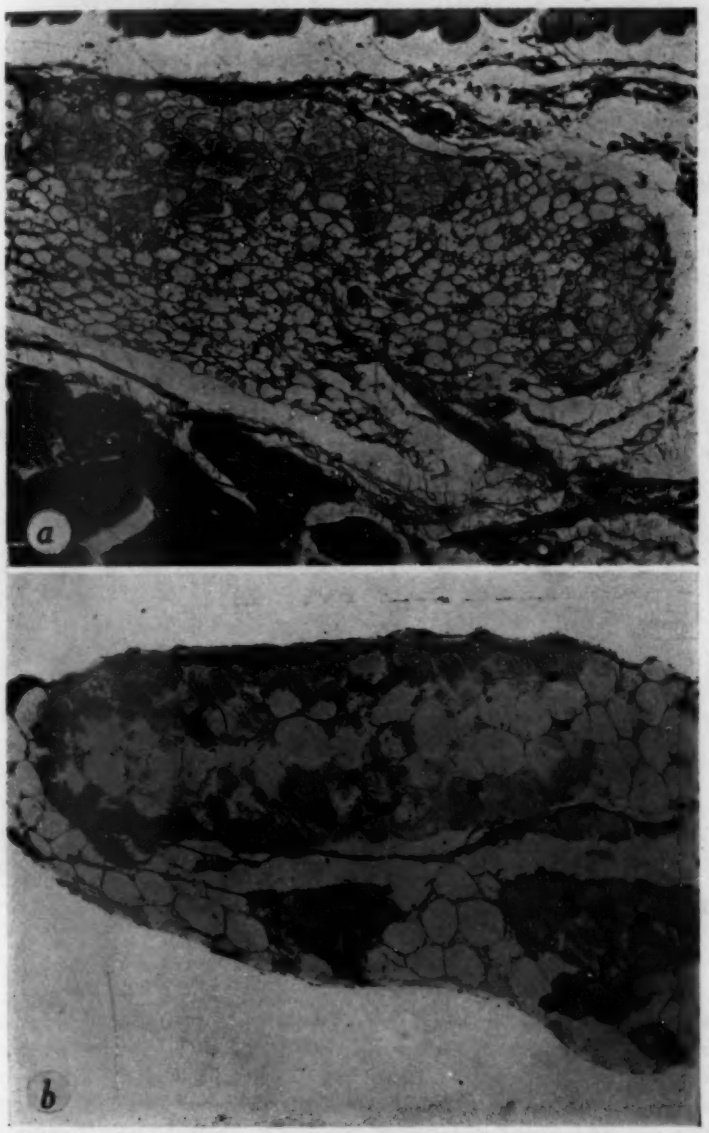

Fig. 2.—(*a*) Section from the anterior thoracic wall showing sites of fat necrosis associated with graphite-filled sternal lymph channels. (*b*) Sites of necrosis associated with graphite-filled lymph channels from the adipose tissue around the inferior vena cava.

be filled with graphite, and closely associated areas of fat necrosis were observed in the adjacent fat as illustrated in figure 2 *a*. A similar relationship existed between

the lymph channels and areas of necrosis in the fat about the inferior venae cavae (fig. 2 *b*). The mediastinal lymph nodes were found to be filled with both free and phagocytosed graphite particles and often necrotic areas were observed in the fat surrounding these nodes (fig. 3).

After the studies described in this paper had been completed, a case of acute pancreatitis was encountered at the Mayo Clinic. The patient died, and at necropsy evidence of abundant fat necrosis was found in both the peritoneal and the thoracic cavity. The distribution of the sites of disseminated fat necrosis was similar to that produced in the animals studied, and therefore the pertinent points of this case were included to demonstrate more conclusively that lipase is transmitted through the lymph channels.

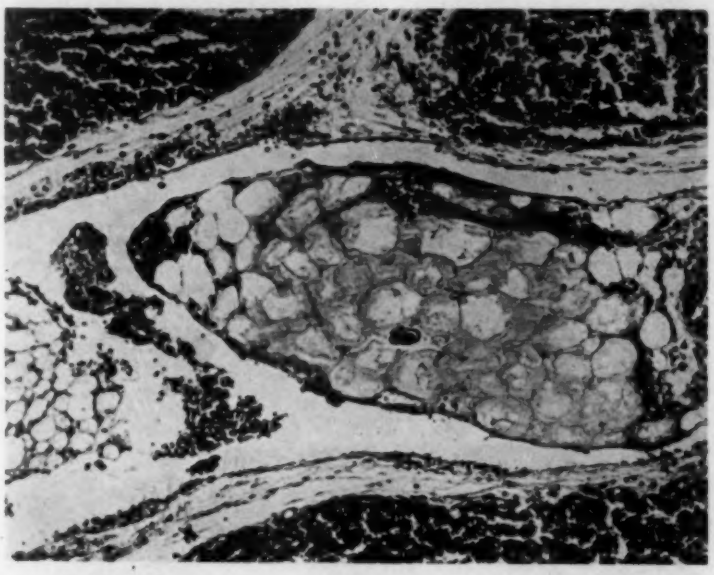

Fig. 3.—Site of fat necrosis lying between graphite-filled mediastinal lymph nodes. Higher magnification showed graphite particles within the saponified lesion.

REPORT OF A CASE

A white housewife 37 years old was admitted to the clinic and was hospitalized immediately. She had had recurrent attacks of severe epigastric pain and pain in the lower part of the thorax. Exploratory operation was carried out because it was suspected that a stone was present in the common bile duct. The patient failed rapidly after operation, however, and died on the third postoperative day.

At necropsy 500 cc. of blood-tinged fluid was found in the peritoneal cavity. Numerous small regions of fat necrosis were found on the surface of the pancreas and in the peripancreatic fat. Evidence of fat necrosis was also present in the omentum, the mesenteries, at the hilus of the liver and that of the spleen and along the lesser curvature of the stomach. The anterior surface of the head of the pancreas presented a region (6 by 2 by 3 cm.) in which hemorrhagic pancreatic necrosis had occurred.

The pleural cavities contained no excess of fluid. Sites of fat necrosis were found scattered over the pleural surface of the diaphragm and to a less extent in the parietal pericardial fat. Similar lesions were observed on the external surface of the thoracic portion of the esophagus.

The fat along the peritoneal surface of the diaphragm presented necrotic spots. In 1 instance the foci appeared to be distributed along the perivascular lymph channels of a small artery and vein (fig. 4 *a*).

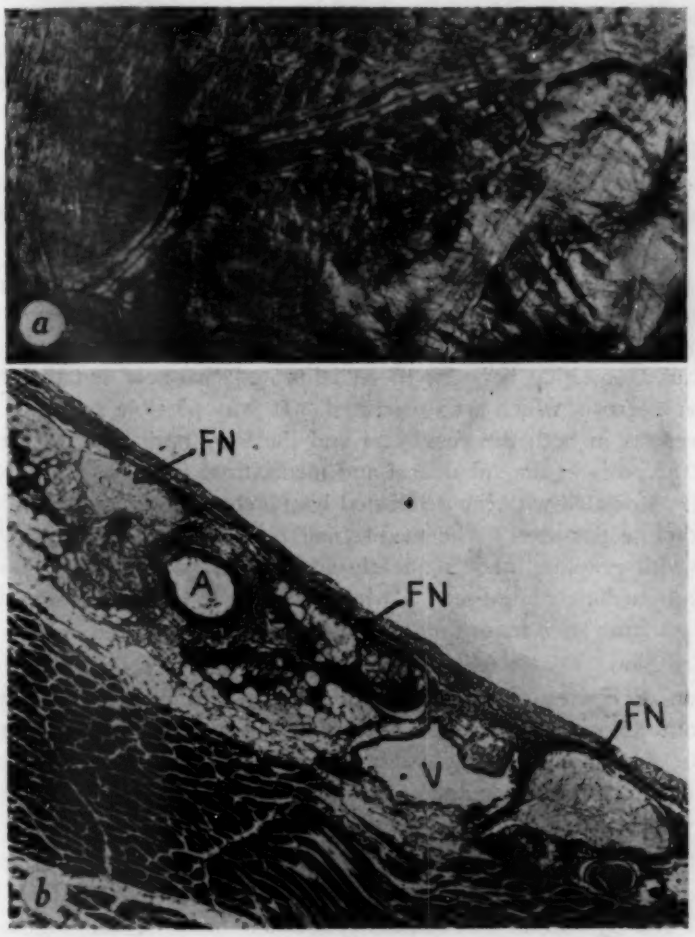

Fig. 4.—(*a*) Peritoneal surface of the diaphragm showing evidence of fat necrosis in the region of the perivascular lymph channels of a small artery and vein. (*b*) Section across small artery (*A*) and vein (*V*) shown in *a*. Typical evidence of fat necrosis (*FN*) may be seen in the region of the perivascular lymph channels.

Microscopic examination of sections of this portion of the diaphragm showed evidence of typical fat necrosis in the region of the perivascular lymphatics (fig. 4 *b*).

The pathologic diagnosis was chronic pancreatitis with extensive fat necrosis.

COMMENT

The evidence presented in this study seems to lend strong support to the hypothesis that lipase is transmitted by means of the lymph channels in so-called pancreatic and peripancreatic fat necrosis.

It has been shown in studies of animals that intraperitoneal injection of lipolytic substances or damage of the pancreas and its duct system will cause necrosis not only in the fat of the peritoneal cavity but frequently also in the subpleural, pericardial and mediastinal fat. A similar distribution of sites of fat necrosis has been noted at necropsy in cases of pancreatic disease recorded in the literature and was seen at necropsy in 1 case at the Mayo Clinic.

Higgins and Graham demonstrated that in dogs the peritoneal and thoracic lymphatic systems are continuous by injecting intraperitoneally a suspension of finely particulate graphite. A somewhat similar lymphatic continuity was said to exist in human beings.

In this study, the graphite suspension mixed with pancreatin and injected intraperitoneally in rats showed not only continuity between the peritoneal and thoracic lymphatic channels but also a close and constant association between these delineated channels and the sites of the fat necrosis which was produced. It was possible to bring about fat necrosis in both the peritoneal and the intrathoracic fat. The sites of fat necrosis in the subpleural and mediastinal fat were not only intimately associated with the delineated lymph channels but were infiltrated by graphite particles. The mediastinal lymph nodes were consistently filled with graphite, and frequently sites of fat necrosis were observed in the immediately adjacent fat, whereas the fat a few millimeters distant was free from such lesions.

The blood vessels were examined as a possible route of the transmission of lipase. They were found to contain no graphite, and no constant association between them and the sites of fat necrosis was noted. Although it is impossible to exclude the blood vessels entirely as a factor in the transmission of lipase, it seems improbable that they were concerned.

Since the diaphragm presented no grossly visible apertures and the thoracic cavities contained no free fluid, the possibility that lipase passes directly into the thoracic cavity seems to be ruled out. Apparently, therefore, the only probable route that lipase could have taken was that through the lymph channels.

A case of chronic pancreatitis in which the associated fat necrosis was disseminated to the intrathoracic fat has been included in this study. The distribution of the sites of fat necrosis was essentially similar to that observed in the rats and it seemed difficult to explain the presence of

these lesions within the thoracic cavity except by the hypothesis that lipase was transmitted by means of the lymphatics.

SUMMARY

Intraperitoneal injections of a mixture of a solution of pancreatin and a suspension of finely particulate graphite were made in 25 large white rats. At necropsy multiple areas of fat necrosis were found in both the abdominal and the thoracic cavities, closely associated with graphite-delineated lymph channels. The hypothesis that lipase is transmitted by the lymph channels in cases of disseminated pancreatic fat necrosis following pancreatic injury and disease is given strong support.

STUDIES OF THERMAL INJURY

IV. An Exploration of the Casualty-Producing Attributes of Conflagrations; Local and Systemic Effects of General Cutaneous Exposure to Excessive Circumambient (Air) and Circumradiant Heat of Varying Duration and Intensity

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IN PREVIOUS studies [1] in this series an investigation was made of the factors which determine the extent to which the energy of environmental heat is transferred to and through the skin, of the rate at which heat is accepted by the skin and at which the temperature of the skin rises, of the time–tissue temperature thresholds at which cutaneous injury occurs and of the sequence of gross and microscopic changes that occur in the skin incident to hyperthermic injury. The skin was exposed locally and directly to a running stream of hot water as the most readily controlled means of applying heat. Although certain predictions were made as to the effects of circumambient [2] and circumradiant heat, no experiments were reported in which animals were actually exposed under such conditions.

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The work described in this paper was done under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development of the National Research Council and the President and Fellows of Harvard College, who assume no responsibility for the accuracy of the statements contained herein.

1. (*a*) Henriques, F. C., Jr., and Moritz, A. R.: Studies of Thermal Injury: I. The Conduction of Heat to and Through the Skin and the Temperatures Attained Therein; a Theoretical and an Experimental Study, Am. J. Path., to be published. (*b*) Moritz, A. R., and Henriques, F. C., Jr.: Studies of Thermal Injury: II. The Relative Importance of Time and Surface Temperature in the Causation of Cutaneous Burns Am. J. Path., to be published. Moritz, A. R.: Studies of Thermal Injury: III. The Pathology and Pathogenesis of Cutaneous Burns; an Experimental Study, Am. J. Path., to be published.

2. Throughout this report "circumambient" is used to denote heat that is available to the skin via conduction and convection of air.

It was realized that many, if not the majority, of severe thermal casualties occurring in man, particularly those that occur incident to military operations, result from exposures in which the skin is in contact with air rather than with water. It was obviously desirable then to acquire the kind of data that would make it possible to estimate man's tolerances, both local and systemic, of thermal exposures in which heat is transferred to the body through an envelope of air and to investigate the mechanisms by which such exposures cause disability and death.

AN EXPLORATION OF THE CASUALTY-PRODUCING ATTRIBUTES OF CONFLAGRATIONS

In order to acquire a certain amount of general or orienting information concerning the thermal and chemical attributes of conflagrations, the following pilot experiments were undertaken. It was intended to observe the rate, the magnitude and the duration of the changes that occurred in the temperature, as well as the changes in the oxygen, the carbon dioxide and the carbon monoxide content of the atmosphere, incident to the burning of gasoline in closed and in ventilated spaces and to assess the relative importance of these various changes in the production of casualties.

EXPERIMENTAL PROCEDURE

Gasoline was burned in a fireproof room having a capacity of 14.4 cubic meters. The construction of the room was such that it could be either closed or ventilated at will. The fuel was poured into shallow metal pans that completely covered the floor, which measured 1.6 by 3 meters. Approximately 4 liters of gasoline was burned during each conflagration.

Method of Measuring Temperature.—In order to measure accurately the anticipated rapid changes in temperature, 40 gage spot-welded iron-constantan thermocouples were suspended in the center of the chamber, and the resulting thermoelectric potentials were amplified by means of an electronic optical bridge circuit,[3] which was capable of amplifying a 1 millivolt input to a 5 milliampere output in less than 0.2 second. Since this amplifier is a null point instrument, it was independent of all the electronic tube characteristics, of the intensity of the light beam focused on the photocell (RCA 920, split cathode) and of the input resistance of the thermocouple leads. Two such amplifiers were constructed. Two recorders were used. One was an Esterline-Angus recording ammeter (5 milliampere full scale) with a response time of 0.5 second. The other was a General Electric photoelectric recording milliammeter with a response time of 0.2 second. Both recorders had 12 inch (30 cm.) per minute chart drives. By means of a selector switch the sensitivities of the amplifiers were usually set so that a 40 millivolt input produced full scale deflections of the recording pen.

3. Gilbert, R. W.: Rev. Scient. Instruments **7**:41, 1936. Muller, R. H.; Garman, R. L., and Droz, M. E.; Experimental Electronics, New York, Prentice-Hall, Inc., 1942.

Method of Obtaining Samples of Atmosphere for Gas Analysis.—Three long tubes, each having an internal diameter of 2 mm., extended from the outside to the center of the conflagration chamber. These tubes passed through the wall at the bottom, the middle and the top of the room. Three hundred cubic centimeter samples were withdrawn as desired by attaching evacuated flasks with ground joints to the ends of these tubes. The gas samples obtained in this manner were analyzed for oxygen, carbon dioxide and carbon monoxide by means of a standard Orsat apparatus.

TEMPERATURES DEVELOPED DURING GASOLINE CONFLAGRATIONS

Unventilated Conflagrations.—In these experiments the depletion of oxygen resulted in extinction of the conflagration in about thirty seconds after ignition of the gasoline. Approximately half of the gasoline contained in each pan remained unburned. When the door was opened after the premature extinction of the fire, the room was found to be filled with dense black smoke, and there was a strong odor of gasoline.

Figure 1 *A* shows continuous records of the temperature, provided by the two thermocouples, one of which was hung midway between the floor and the ceiling in the center of the 3 meter high conflagration chamber, and the other, about 0.9 meter above the floor.

Owing to rapid convection currents, the upper thermocouple reached higher temperatures than did the lower. The sharp peaks of the temperature curve of the upper thermocouple are also due to convection currents. The average temperatures recorded by the two thermocouples over a thirty second period were approximately the same, namely, about 500 C. At the termination of the combustion the ambient temperatures fell rapidly and uniformly. The curves shown in figure 1 are typical of all experiments in which the conflagration was unventilated.

Ventilated Conflagrations.—Figure 1 *B* shows a continuous recording of the temperature of a thermocouple which was situated about 1.5 meters above the floor during a conflagration in which ventilation sufficient to maintain complete combustion was provided.

The temperatures obtained were about the same as those recorded during unventilated conflagrations. The duration of the high temperature plateau depended on the length of time that the door was left open. In the experiment in which the record shown in figure 1 *B* was made, the door was left open for fifty seconds.

PRELIMINARY OBSERVATIONS ON ANIMALS EXPOSED TO THE HEAT AND COMBUSTION PRODUCTS OF BURNING GASOLINE

Adult dogs (6 to 8 Kg.) and young pigs (7 to 12 Kg.) were exposed in various ways to burning gasoline. The animals were anesthetized by an intraperitoneal injection of pentobarbital sodium and fastened by asbestos tape to an iron frame situated in the center of the conflagration room 54 inches (1.5 meters) above the floor. The principal data pertaining to these experiments are included in table 1.

Combined Cutaneous and Respiratory Exposure.—Animals 1 and 2 were exposed to the full effects (cutaneous and respiratory) of the burning gasoline. Throughout the entire exposure of animal 1 the door of the conflagration chamber remained closed. The fire burned out in about thirty seconds, because of insuf-

ficient oxygen. The average temperature of the air surrounding the animal during this period was 320 C. The animal was allowed to breathe the atmosphere of the unventilated room for five minutes after the fire was extinguished.

Samples of the atmosphere were taken for gas analyses as soon as the fire had burned out. The mean concentration of carbon monoxide in the atmosphere was 0.8 per cent, and the concentration of oxygen was 14.6 per cent. The carbon

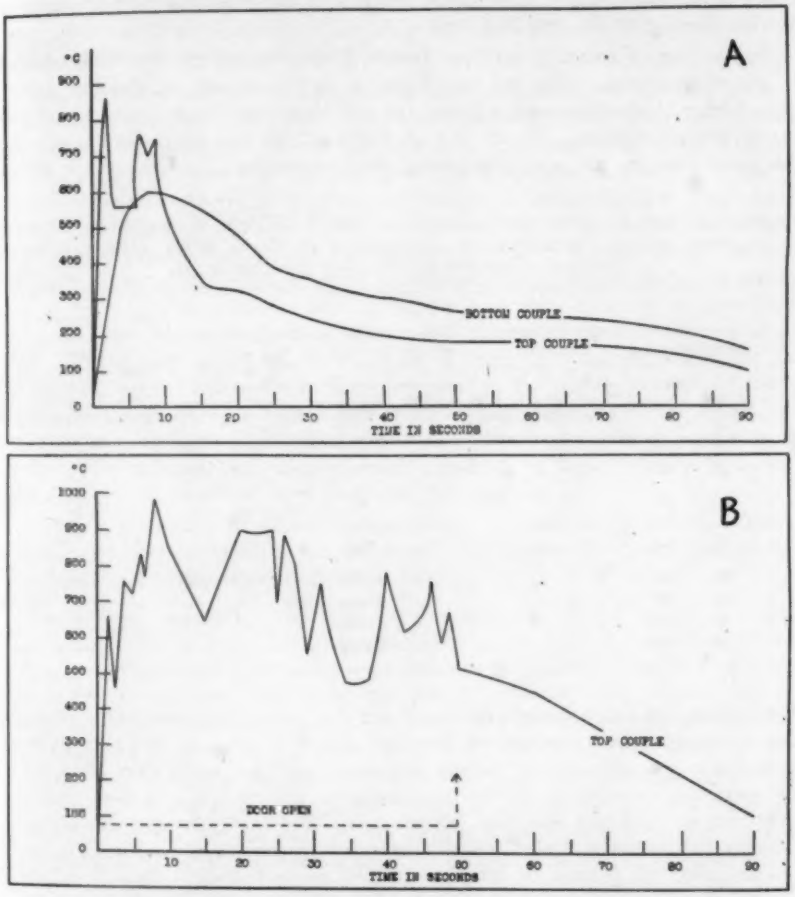

Fig. 1.—Temperature recorded continuously during burning of gasoline in a rectangular combustion chamber (6 by 10 by 10 feet [about 2 by 3 by 3 meters]). During the recording in *A* there was no ventilation of the room. Two thermocouples were used, one 5 feet (1.5 meters) and the other 3 feet (about 1 meter) above the floor level. The distance from floor to ceiling was 10 feet. During the recording in *B*, the room was ventilated for fifty seconds. One thermocouple was used, which was 5 feet above the floor.

monoxide saturation of a sample of the animal's blood taken five minutes later was 30 per cent. Although there was no indication that the fire had resulted in a dangerously low oxygen or a dangerously high carbon dioxide concentration.

it did appear likely that the animal would have died of carbon monoxide poisoning if it had remained much longer in the unventilated room.

Although animal 1 had been severely burned, it did not present early shock, required several postexposure injections of pentobarbital sodium to keep it quiet and was beginning to become restless with returning consciousness when killed six hours later. Its air passages contained an excessive amount of mucus, but there was neither clinical nor pathologic evidence of significant thermal or chemical injury of larynx, air passages or lungs, which is in accord with previous findings of Moritz, Henriques Jr. and McLean.[4]

In the case of animal 2 the door remained open during the first forty seconds of the conflagration, with the result that a larger amount of gasoline burned and a higher temperature was achieved and was maintained for a longer period than in the first experiment. At the end of forty seconds the door was closed, with the result that the fire was extinguished soon thereafter. Samples of the atmos-

TABLE 1.—*Data on Nine Experiments in Which Effects of Excessive Temperatures and of Inhalation of Combustion Products Were Observed in Animals Exposed to Gasoline Conflagrations*

Animal and Experiment No.	Conflagration		Site of Thermal Exposure			Inhalation of Combustion Products		Composition (%) of Air Immediately After Fire			Fate of Animal		Blood After Exposure: CO Saturation, %
	Average Time, Sec.	Temp., C.	Body and Face	Body Only	Face Only	With Fire	After Fire	O_2	CO_2	CO	Dead in 15 Min.	Survival for Hrs. or Days	
1	30	320	+	..	..	+	5 min.	14.6	4.0	0.8	..	+	30
2	40	600	+	..	..	+	No	16.2	4.0	0.8	+	..	7
3	30	400	..	+	..	No	No	....	...	...	..	+	..
4	30	370	..	+	..	No	No	....	...	...	..	+	..
5	75	700	..	+	..	No	No	....	...	...	+	..	..
6	30	350	..	..	..	No	5 min.	14.7	4.6	1.0	..	+	6
7	30	350	..	..	+	+	4 min.	15.7	3.5	0.3	..	+	Trace
8	30	450	..	..	+	+	4 min.	16.1	3.6	0.7	..	+	37
9	30	500	..	..	+	+	2 min.	....	...	...	..	+	32

phere were then taken for gas analyses, and the animal was removed. This dog was moribund when removed to the open air. In view of the fact that the atmospheric concentration of carbon monoxide was similar to that observed in the preceding experiment, it was surprising to find that the carbon monoxide saturation of the blood was only 7.0 per cent. The explanation of this disparity probably lies in the fact that animal 1 breathed the atmosphere of the conflagration chamber for a total of between six and seven minutes, whereas animal 2 was moribund at the end of two minutes.

Two factors may have contributed to the extremely rapid death of dog 2. One is systemic hyperthermia caused by overheating of the blood as it circulated through the extensive superficial network of subcutaneous vessels. The other is respiratory obstruction due to pharyngeal edema. That obstruction of respiration may have contributed was indicated by the severe burning of mouth and pharynx accompanied with what appeared to be obstructive edema of the latter.

4. Moritz, A. R.; Henriques, F. C., Jr., and McLean, R.: Am. J. Path. **21**:311, 1945.

The trachea and bronchi contained abundant mucus mixed with carbon particles. The lungs were hyperemic.

The results of the first two experiments in which animals were exposed to burning gasoline indicated that even in circumstances that were particularly favorable to the production of carbon monoxide and to the exhaustion of oxygen, the concentration of these gases was not sufficiently altered to cause rapid asphyxia. Although the results of two experiments are not construed as evidence that neither fatal anoxia nor fatal carbon monoxide poisoning is likely to result from a gasoline fire, they do indicate that such exposures can be rapidly fatal from thermal injury alone.

Cutaneous Exposure.—The next three experiments shown in table 1 were undertaken to ascertain the effect of protecting the respiratory tract against heat and combustion products during the time that the body was being exposed. To investigate this question, animals 3, 4 and 5 wore a tight-fitting asbestos-covered mask through which a continuous stream of unheated air was circulated during their exposure to heat. The first 2 animals of this series (3 and 4) were exposed to an unventilated conflagration of about thirty seconds' duration and average atmospheric temperatures of 400 and 370 C., respectively. Although both animals showed extensive burning of the skin, they survived the immediate effects of heat and were in reasonably good condition when killed six hours later. In the case of animal 5, the door of the room was left open for the first minute of the fire, and for sixty-five seconds the temperature of the room was in excess of 400 C. Within fifteen seconds after the door was closed, the fire went out, and the animal was removed. This animal died immediately on reaching the open air and showed severe burning of all of the body surface except where the skin had been protected by the mask.

These experiments provided evidence that a relatively brief (seventy-five seconds) exposure of the skin to a sufficiently high temperature could cause almost immediate death independently of other factors.

Respiratory Exposure.—The last four experiments shown in table 1 were undertaken in an attempt to investigate further the effects produced on animals by the breathing of the combustion products of a gasoline conflagration. In each experiment the door was kept closed throughout the entire conflagration. By this procedure, postconflagration mixing of outside air with the combustion products was reduced to a minimum. The skin of the body was protected against excessive overheating by enclosing the animals to the neck in a heavy asbestos sack. With the exception of dog 6 the animals were free to breathe the burning gases and hot air during the fire as well as the smoke which remained in the chamber after the fire. Dog 6 breathed outside air circulated through the mask during the fire, and as soon as the temperature in the room had dropped to 200 C., the mask was detached by remote control and for the next five minutes only the hot smoke and air of the combustion chamber were available for respiration.

None of these 4 animals showed either clinical or pathologic evidence of thermal injury of the air passages or the lungs. Two of them (6 and 7) may have held their breath throughout most or all of the period of exposure. That animals 7 and 8 breathed during some of the time that they were in the combustion chamber is indicated by their carboxyhemoglobin concentrations of 37 and 32 per cent, respectively. It is possible, of course, that even these 2 animals held their breath during the conflagration and acquired their carbon monoxide by breathing during the interval between the time that the fire went out and

the time that they were removed from the chamber. A more extensive investigation of the problem of thermal injury of the lungs and air passages has already been reported.[4]

The most important information gained from these exploratory experiments was the observation that animals as large as dogs and pigs when exposed to this kind of conflagration for more than thirty seconds may receive injuries that are almost immediately fatal. The fatality was not necessarily contributed to by asphyxia, carbon monoxide poisoning or inhalation of flame. It was apparent that rapid death may result from systemic disturbances caused by the impact of heat energy on the surface of the body.

LOCAL AND SYSTEMIC EFFECTS IN ANIMALS WHOSE GENERAL CUTANEOUS SURFACE WAS EXPOSED TO CIRCUMAMBIENT (AIR) AND CIRCUMRADIANT HEAT OF VARYING DURATION AND INTENSITY

A series of more adequately controlled experiments was now undertaken to investigate the quantitative relationships of temperature, time and casualty production and the mechanisms responsible for casualties during general cutaneous exposure to circumambient and circumradiant heat.

EXPERIMENTAL PROCEDURE

Healthy young pigs were used. Each animal, previously clipped and anesthetized by an intraperitoneal injection of pentobarbital sodium, was fastened on a platform in the manner shown in figure 2 and a preheated oven lowered over it. In most of the experiments the snout of the pig protruded through an aperture in the bottom of the platform. There were two advantages to this method of exposure, one being that the respiratory tract was protected and the other being that it was possible to determine the time of death of any animal that succumbed during the period of exposure.

The Apparatus.—The source of heat was a bottomless oven constructed of iron and firebrick and having a capacity of approximately 1,100 liters. The box weighed 2,700 Kg., and its internal measurements were 89 by 91 by 130 cm. Four chromel alumel (10 gage) thermocouples welded onto the inside iron plates of the box provided information as to the temperature of the source of heat during the period of preexposure heating as well as during the period that the animal was being exposed. For preheating, the box was lowered into a vertical gun-annealing furnace,[5] and when it had become thoroughly heat soaked and was at a temperature slightly higher than that to which the animal was to be exposed, it was quickly withdrawn from the furnace by an overhead crane and lowered over the platform on which the animal was suspended. The interval required for the descent of the box from the top of the tripod to the floor of the platform was between three and four seconds.

5. These facilities at the Watertown Arsenal, Watertown, Mass., were made available by the War Department.

The platform supporting the tripod on which the pig was suspended was elevated 75 cm. above the floor and was covered by a layer of dry sand. In addition to the aperture to accommodate the snout of the animal there were other openings in the platform through which wires could be passed to the temperature-recording equipment.

Three 28 gage iron-constantan thermocouples connected in parallel were fastened to the surface of the animal in such a way that the junctions were separated from the skin by a distance of between 2 and 5 cm. These provided for a continuous recording of ambient temperature.

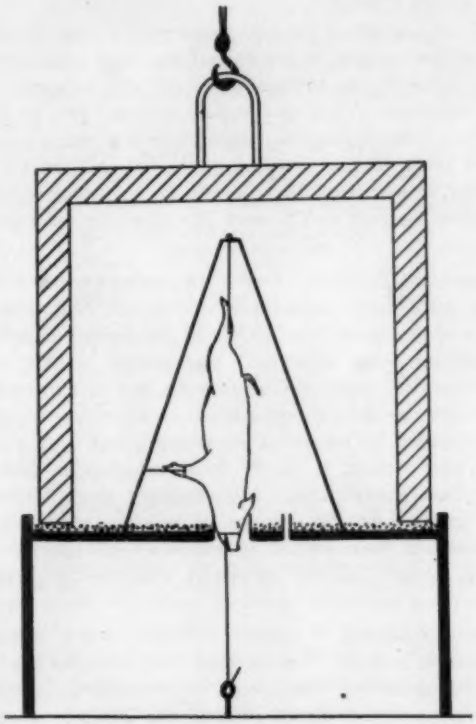

Fig. 2.—Method of exposing the experimental animal to hot air and radiant heat at Watertown Arsenal. A heavy oven constructed of iron and firebrick was preheated in a gun-annealing furnace and lowered over the platform.

Recording of Temperatures.—Rectal temperatures were taken routinely. In some experiments a rectal thermocouple provided for a continuous record. In others the temperature was taken by thermometer before and at intervals after exposure. On several occasions the postexposure temperature of the blood of the right auricle was taken for comparison with that of the rectum.

In a number of experiments a 28 gage iron-constantan thermocouple contained in a venipuncture needle was inserted into the dermis to record the temperature of the subepithelial connective tissue during and after exposure.

The temperature of the air of the exposure chamber was determined in different parts of the chamber. The values given in the text for ambient temperature

refer to the mean temperature of the air in which the animal was enveloped. The thermocouples by which the ambient temperature was measured were placed in approximately the same positions in relation to the animal in all experiments. One was fastened to the skin just below the base of the tail and one on each side of the midportion of the body. It was regularly observed that the mean ambient temperature was approximately 20 per cent lower than that measured by the thermocouples incorporated in the wall of the exposure chamber. Although the rate of cooling of the exposure chamber (and of the air contained by it) varied according to the magnitude of the initial difference between its temperature and that of the room, the drop was never in excess of 5 per cent in experiments lasting less than fifteen minutes.

On account of the convection currents that resulted from the difference between the temperature of the surface of the animal and that of the air surrounding it, the temperatures recorded in various parts of the exposure chamber showed remarkably little variation. Thus in the midhorizontal axis of the chamber there was less than 5 per cent difference in temperature between a point 15 cm. internal to the wall and a point 15 cm. external to the animal. In the midvertical axis there was less than 15 per cent difference in the temperature of the air between a point 15 cm. below the roof and a point 15 cm. above the floor of the exposure chamber.

Measurement of Heat Transfer.—Under the conditions in which these exposures were made there were three mechanisms by which heat could be transferred from the hot walls of the box to the surface of the animal, namely, air conduction, air convection and infra-red radiation. The energy transferred by conduction and convection is hereafter designated as ambient, and that transferred by radiation as radiant. Although the relative importance of these two types of heat transfer can be directly computed by means of equations 1 and 2 of a previous report[1a] in this series, it was decided to verify those calculations under the conditions that prevailed in these experiments. Unfortunately, direct determinations of the ambient and the radiant caloric uptakes of animals were not feasible, and it was necessary to obtain these values by means of calorimeters which had been suspended in the center of the exposure chamber preliminary to animal experimentation.

The calorimeters consisted of copper cylinders which measured 2.5 cm. in diameter and 5 cm. in length. One of each pair of cylinders was gold plated, and the other was blackened with colloidal graphite (Aquadag). Thus, the former measured only ambient energy, whereas the latter determined both ambient and radiant energy.

The caloric uptake rate of the calorimeters was readily calculated from their known heat capacity and surface area and the experimentally determined rate of temperature rise as measured by an iron-constantan thermocouple soldered within the calorimeter. Owing to the discrepancy in size between these calorimeters and the pigs (about 30 by 75 cm.), it was necessary to multiply the ambient calorimetric measurements by a numerical factor[6] equal to 0.5. Since the skin is known to be nearly a perfect black body for the radiation emitted under these experimental conditions and since the dimensions of the exposure chamber were large with respect to those of the animal, the radiant caloric measurements are directly applicable.

Actually, these data, so corrected, apply to a metallic cylinder of dimensions similar to those of a pig. In view of the fact that it has been shown that under the conditions of experimentation these data would be equally applicable to

smooth and rough and to metallic and nonmetallic surfaces,[6] it is believed that they represent a true estimation of the caloric uptake of pig skin.

The data given in table 2 are an estimation of the radiant and ambient caloric uptake rate per square centimeter per minute of pig skin when the surface temperature is 35 C. It is obvious that during the heat exposure the surface temperature increases with time, which results in a corresponding decrease in the rate of caloric uptake. For cutaneous surface temperatures not greater than 60 C. the rates of caloric uptake are directly proportional to the difference between the temperature of the surrounding air and that of the surface of the animal. Thus, for surface temperature below 60 C. the rates of caloric uptake as a function of temperature of the surface of the skin can be computed from these data. Further examination of table 2 shows that the infra-red radiation from the inside walls of the box was the principal source of the heat energy absorbed by the animals. Under conditions that produced an air temperature of 70 C.

TABLE 2.—*Estimated Caloric Uptake of the Pig When the Temperature of the Surface of the Skin is 35 C.*

Air Temperature, C.	Caloric Uptake in Calories per Sq. Cm. per Min.			Per Cent of Total Contributed by Radiant Caloric Uptake
	Nonradiant (Ambient)	Radiant *	Total	
70	0.2	0.2	0.4	50
100	0.5	0.6	1.1	55
150	1.0	1.4	2.4	58
200	1.7	2.6	4.3	61
250	2.2	4.2	6.4	66
300	3.0	6.2	9.2	68
350	3.8	9.8	13.6	72
400	4.5	17.0	21.5	79
450	5.5	24.0	29.5	81
500	6.5	35.0	41.5	85

* Because of the difference between the air temperature and the source temperature when the animal is placed in the exposure chamber, the data concerning radiant heat refer to a source temperature 20 per cent in excess of the tabulated ambient temperature.

this contribution was 50 per cent, while at 500 C. it was 85 per cent. These percentages remained nearly invariant throughout the entire time of a given heat exposure. As previously indicated, these values for the nonradiant and the radiant contribution to the caloric uptake rate can be directly computed (equations 1 and 2)[1a] and, if this is done, it will be found that they agree with the experimental values to within 15 per cent.

EFFECTS ON ANIMALS

The results of 71 individual exposures of pigs are shown in figure 3. It was at first intended to present in this chart only the data derived from 49 experiments in which pigs of uniform weight (7 to 18 Kg.) had their general cutaneous surface about 90 per cent exposed to heat. The additional 22 experiments included those in which large animals (in excess of 15 Kg.) were used, those in which hot air was breathed during the time that the skin was being exposed and those

6. McAdams, W. H.: Heat Transmission, New York, McGraw-Hill Book Company, Inc., 1942.

in which the animals were anesthetized after rather than before exposure. When it was found that there were no significant differences in the experimental results that could be related to the body weights of the animals (7 and 32 Kg.) or to anesthesia, it was decided to present all experimental data in one chart.

The temperature and the duration of each exposure are indicated by the position of the individual experiment on the grid. The vertical points of reference

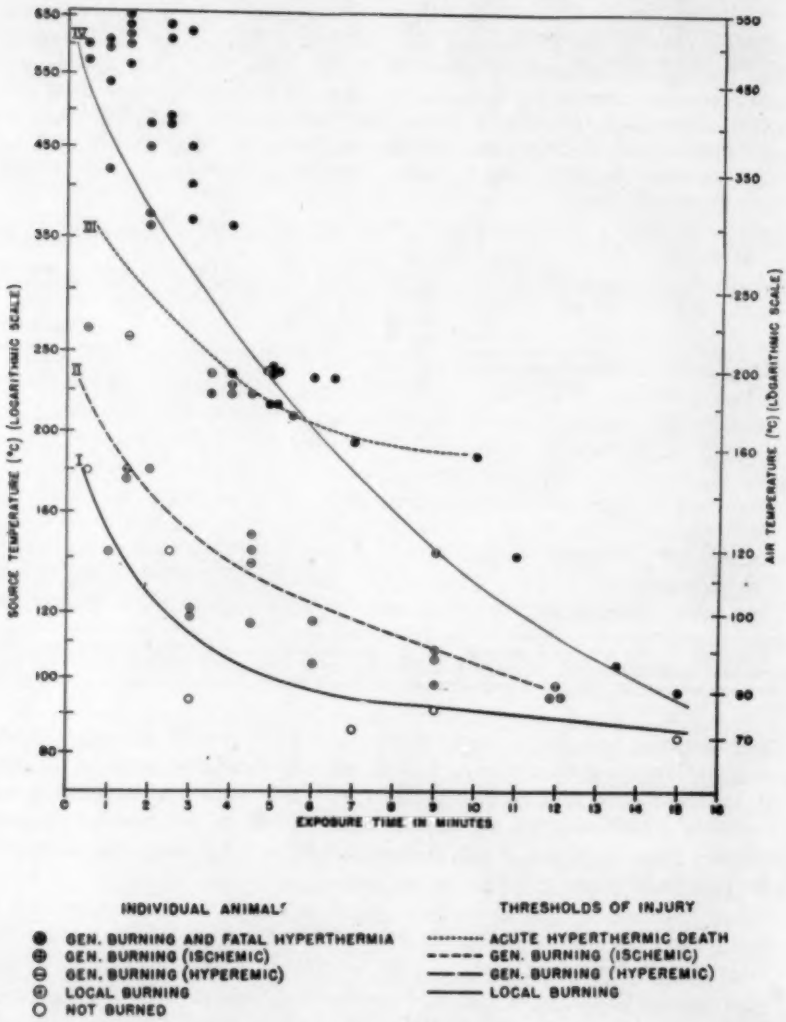

Fig. 3.—Graph showing results of 71 experiments in which pigs had their general cutaneous surface exposed to ambient and radiant heat in an oven. Each experiment is depicted by a circle. The duration and the temperature of the exposure are indicated by the position of the circle in the grid. The effect of the exposure on the pig is shown by the character of the circle. The curved lines traversing the grid depict the approximate thresholds at which varying degrees of cutaneous and systemic injury occurred.

on the left are in logarithmic progression and represent the internal surface temperature of the exposure chamber, whereas those on the right represent the corresponding ambient temperature in the vicinity (within 5 cm.) of the animal. The horizontal axis indicates the duration of the exposure.

In addition to the experiments shown in figure 3, several pigs were exposed for periods longer than fifteen minutes at source temperatures of 90 C. or higher. At 80 C. pigs survived exposures as long as forty-five minutes without cutaneous burning and without the development of fatal hyperthermia. After forty-five minutes at approximately 90 C. fatal hyperthermia developed, and although the skin was intensely hyperemic, there was no evidence that irreversible epidermal injury had been sustained.

It may be seen that the experiments fall into three main groups so far as their effects on the pigs were concerned: Some animals were unharmed and showed neither superficial nor systemic evidence of injury, others sustained cutaneous injury but with insufficient systemic disturbance to result in early collapse and death, and still others died during or within thirty minutes after the exposure.

All animals underwent a rise in body temperature as a result of exposure, and so far as the blood temperature was concerned, the maximum rise usually coincided with or was attained within a few minutes after the conclusion of the exposure. If death was to result from systemic hyperthermia, it usually occurred soon after the maximum body temperature was reached—almost invariably within thirty minutes. If an animal survived long enough after exposure for its body temperature to fall to 40 C. or lower, it characteristically survived many hours and either recovered or was killed. No example of persistent hyperthermia such as that which commonly follows heat stroke in man was encountered. The relation of systemic hyperthermia and death in pigs will be discussed in greater detail in a later section of this report.

The upper limits of exposures which pigs survived without either cutaneous burning or fatal systemic hyperthermia are indicated by the first line (*I*) that traverses the grid from left to right. Exposures lying below this line failed to cause lasting disturbances, either local or systemic. Exposures lying between the first and second lines characteristically resulted in mild or localized burning. (See figure 4*A*.) The second line (*II*) represents the approximate threshold at which generalized hyperemic burning occurred. (See figure 4*B*.) The third line (*III*) represents the approximate threshold at which the burned skin and subcutaneous tissue underwent ischemic coagulation. (See figure 4*C*.) The skin of most pigs that received exposures above this threshold was pale, and the loss of elasticity of the coagulated superficial tissues resulted in the formation of deep fissures when the extremities were flexed. The uppermost curve (*IV*), which eventually intersects the others in its descent, represents the approximate threshold at which rapidly fatal systemic hyperthermia occurred. Most pigs receiving exposures in excess of this threshold died within a few minutes after the oven had been lifted from the platform. (This period was usually under fifteen and occasionally as long as thirty minutes.)

Comparison of Effects of Hot Air and Hot Water Exposures.—In figure 5 are depicted the temperature-time relationships that were required to produce transepidermal necrosis in hot air exposure and in hot water exposure; in the latter the surface of the skin was maintained at essentially the same temperature as that of the source.[1b] A comparison of the two curves shows that a fifteen minute exposure to water at 48 C. was sufficient to produce approximately the same degree

of injury that resulted from a fifteen minute exposure to circumambient and radiant heat at an air temperature between 75 and 80 C. A one minute exposure to hot water at 53 C. produced about the same degree of injury as resulted from a one minute exposure to ambient and radiant heat at 160 C. It is apparent, therefore, that the surface temperatures responsible for the kind of irreversible injury observed at threshold *II* in figure 3 were considerably lower than the recorded ambient temperatures at which they were produced. In the hot water exposures the change of tissue temperature with time was determined by the rate at which

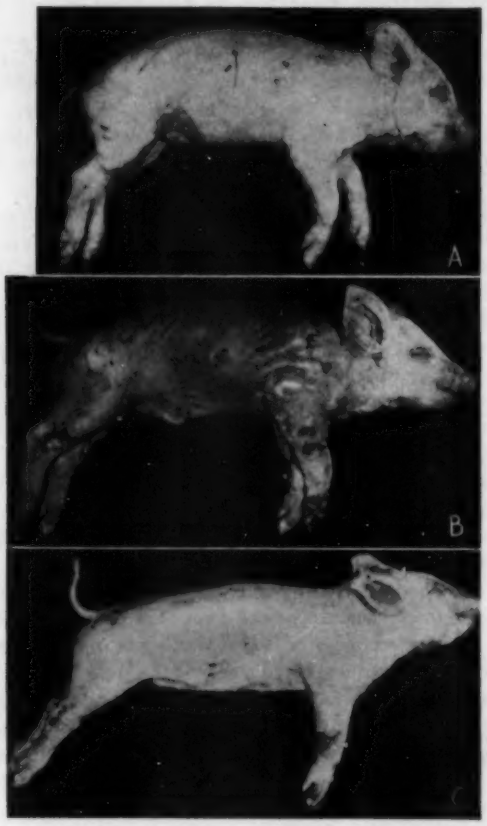

Fig. 4.—*A*, mild, localized burning of the skin of an animal that was exposed for nine minutes at an air temperature of 80 C. (See figure 3). Fatal hyperthemia resulted from an eighteen minute exposure at this temperature.

B, generalized hyperemic burning of an animal that was exposed for four minutes at an air temperature of 180 C. Fatal hyperthermia (43.5 C.) developed, and the animal died within twenty minutes.

C, generalized ischemic burning of an animal that was exposed for five minutes at an air temperature of 200 C. This animal died of systemic hyperthermia (44.5 C.) during the exposure.

heat flowed through the skin, whereas in the oven exposures it was limited by the rate at which heat was transferred to the surface.

Through the application of the general theory of heat flow, the actual time-temperature relationships within the epidermis for both hot water and hot air exposures have been computed and tabulated in a previous report (equation 6; tables 5 and 6),[1a] and it appears that the production of a given degree of thermal injury depends only on the time-temperature relationships within the tissue, irrespective of the source of the heat. Furthermore, it is apparent in the case of circumambient and radiant heat that long before the temperature of the surface of the skin would approach that of the air the animal would have succumbed to generalized hyperthermia.

Probable Effects of Comparable Exposures on Man.—So far as the cutaneous effects of ambient and radiant heat are concerned, the reactions of man and pig should be similar if the time-temperature relationships within the epidermis were the same in each instance. (See figure 4—Henriques and Moritz.[1b]) However, a predictable dif-

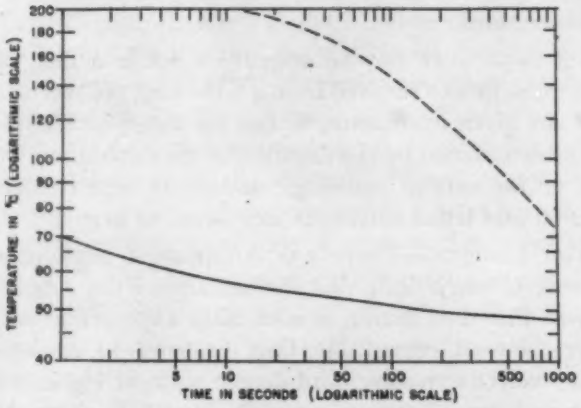

Fig. 5.—The solid curve depicts the time–source temperature relationships requisite to complete transepidermal necrosis when a cutaneous site was exposed to flowing water of constant temperature.[1b] The broken curve shows the time–air temperature relationships (curve II of fig. 3) that produced a similar degree of injury when the cutaneous surface was surrounded by an envelope of radiant and ambient heat (oven experiments).

ference in these relationships during identical heat exposures of this type arises from the fact that sweating of human skin can undoubtedly elevate the time threshold at which cutaneous burning occurs.

That sweating can afford considerable protection in relatively low intensity hot air exposures can be assumed from the fact that man may lose moisture by this mechanism at the rate of approximately a liter per hour. This could result in heat being lost at the rate of between 0.5 and 1.0 calory per square centimeter of cutaneous surface per minute. Heat lost by porcine skin through vaporization of moisture is relatively slight (about 0.1 calory per square centimeter per minute [1b]). Thus, in view of the data concerning caloric uptake (table 2) it seems possible that the time threshold for cutaneous burning is appreciably higher for man

than for the pig at all circumambient and radiant temperatures lower than about 120 C. That such a degree of protection would be afforded at higher air temperatures is unlikely since it would be necessary to assume that sweating was already established at a significant level at the moment of exposure and that all of the sweat excreted was vaporized. No experiments were conducted to establish the quantitative extent to which sweating may be capable of protecting human skin against thermal injury at either low or high ambient and/or radiant temperatures.

It should be emphasized that these data refer only to unclothed animals. It is possible to estimate the degree of protection afforded by articles of clothing by a knowledge of their impedance of the heat reaching the surface of the skin,[6] but since this thermal impedance is so dependent on the physical characteristics of the fabrics involved, on tightness of fit and on the type of heat exposure, further consideration of this problem is not warranted.

Death of Pigs[7].—It may be seen from figure 3 that rapidly fatal physiologic disturbances resulted from a wide range of thermal exposures and that at any given temperature within the range investigated survival or death was determined by the duration of the exposure. Observations were made on the various pathologic and physiologic changes resulting from sublethal and lethal cutaneous exposures to heat.

Pathologic Changes.—There was no apparent relationship between the occurrence of early death and the severity of the cutaneous injury. Some animals that died during or soon after exposure at relatively low temperatures showed remarkably little evidence of cutaneous injury. Others that received extensive third degree burns at higher temperatures survived many hours after exposure and showed no signs of impending death at the time at which they were killed. It was obvious that cutaneous injury per se was not responsible for early collapse and death.

Apart from cutaneous burning there were no significant differences in the pathologic changes observed in animals that died after being exposed to high temperatures for short periods and in those that died after being exposed to lower temperatures for longer periods. The pathologic characteristics of these and other cutaneous burns have been described in detail in another [1c] of this series of reports.

The most constant postmortem finding in all animals that died of hyperthermic shock within thirty minutes after exposure was the small and large foci of hemorrhage disseminated throughout the internal viscera. These were seen most frequently and prominently beneath the endocardium of the right and the left ventricle.

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7. Several goats and dogs received exposures estimated to be lethal or sublethal for pigs, and the impression was gained that their susceptibility to fatal systemic hyperthermia did not differ significantly from that of the pig.

The right auricle was characteristically dilated and filled with dark red, unclotted blood. The impression was gained that the ventricles were more frequently found in the state of contraction after high than after low intensity exposures.

Even in pigs that breathed air so hot that their nasal mucosa was charred, there was no evidence of injury to their bronchial mucosa or lungs. The lungs of pigs rarely showed more than a mild degree of edema, irrespective of the type or the severity of exposure. The moderate to severe pulmonary edema that was observed in dogs and goats bore no relation to the inhalation of heat but was a result of circulatory failure incident to systemic hyperthermia. Fat emboli were usually present in the pulmonary capillaries but not in numbers such as to constitute an adequate explanation of death.

Animals killed twelve to twenty-four hours after sustaining severe cutaneous burns frequently showed severe parenchymatous degeneration of the adrenal cortex, the liver and the renal tubular epithelium. Neither hematoxylin and eosin nor Nissl preparation of the brains of animals dead of hyperthermia disclosed evidence of degenerative or reactive change. It should be borne in mind, however, that hemoglobin casts were sometimes observed in the collecting tubules of the kidneys and that the urine of animals regularly contained large amounts of blood pigment if severe burning was survived by more than a few hours.

Changes in Blood.—Erythrocytes: Examination of severely burned animals regularly showed intravascular hemolysis. That hemolysis was not the determining factor in survival was indicated by its absence in animals that died of hyperthermia following low intensity exposures. A more complete discussion of the relationship between intensity of thermal exposure and hemolysis will be found in number VI of this series of reports.[8]

Examination of wet and dry smears of blood of severely burned animals disclosed microspherocytosis and disintegration of erythrocytes. (See figure 6.) These changes were similar to those observed in the blood of burned human subjects by Shen, Ham and Fleming.[9] They were not observed in the blood of animals that died after low intensity thermal exposures. In severely burned animals there was an increase both in the clotting time and in the fragility of erythrocytes.

Plasma: The observation of turbidity of the plasma together with the findings of small agglomerates of protein and enmeshed cells in wet

8. McLean, R.; Moritz, A. R., and Roos, A.: Studies of Thermal Injury: VI. Hyperpotassemia Caused by Cutaneous Exposure to Excessive Heat, J. Clin. Investigation, to be published.

9. Shen, S. C.; Ham, T. H., and Fleming, E. M.: New England J. Med. **229**:701, 1943.

smears of blood of some fatally burned animals led to a reinvestigation of a phenomenon described by Kabat and Levine.[10] These observers reported that 4 cc. of heated citrated plasma intravenously injected into a cat caused immediate death. After centrifugation of such plasma they found that the supernatant fluid produced no ill effects, whereas death resulted from the intravenous injection of the suspended sediment.

A repetition of the experiments of Kabat and Levine resulted in the observation that blood pressure fell rapidly and that some animals died

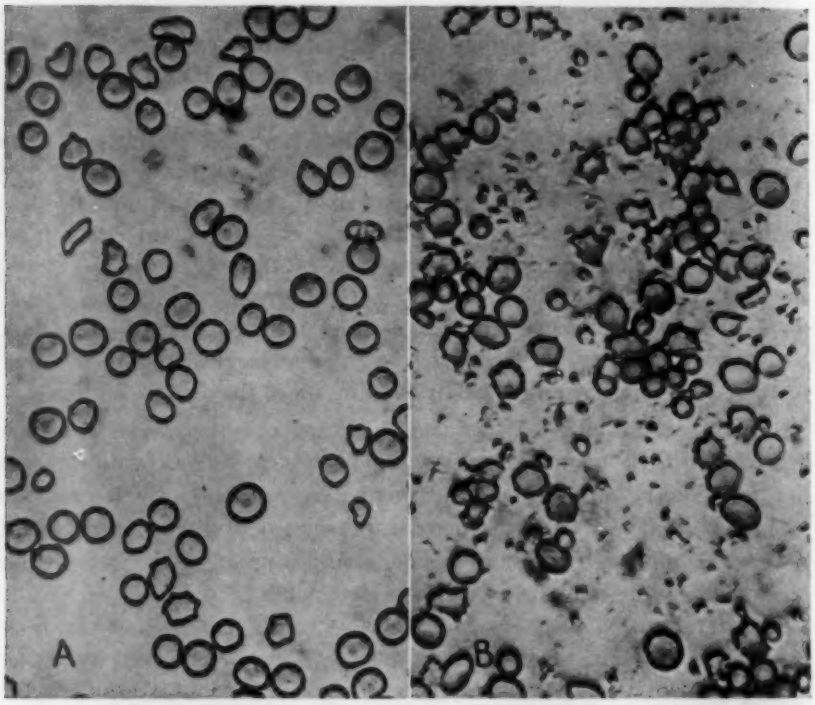

Fig. 6.—Blood smears of pig 856 (9.1 Kg.) before (*A*) and three minutes after (*B*) a five minute exposure to ambient and radiant heat at a temperature of 180 C. The animal received third degree burns of about 85 per cent of the body surface and died three minutes later with a rectal temperature of 43.5 C. Examination disclosed intravascular hemolysis, a plasma potassium concentration of 19.4 milliequivalents per liter and distintegration of erythrocytes as shown in *B*. During the exposure the temperature at the interface between dermis and subdermal fat, recorded by a needle thermocouple, rose to a maximum of 63 C.

in consequence of the intravenous injection of a small amount of heated citrated plasma. However, when heparin was used as anticoagulant instead of citrate, animals tolerated intravenous injection of relatively large amounts of heated plasma without ill effects and without significant

10. Kabat, H., and Levine, M.: Science **46**:476, 1942.

change in blood pressure. Slight lowering of blood pressure was observed in a few animals after injection of heated heparinized plasma or of sediment of heated plasma. No deaths occurred, however, even when amounts as great as 15 cc. were used.

It was concluded that the particulate masses in preheated blood described by Kabat and Levine may be deleterious to a slight degree and in combination with sodium citrate (250 mg. per 10 cc. of blood) may cause death if injected rapidly. It is not believed, however, that these masses contributed significantly to the hyperthermic deaths observed in these experiments.

Perfusion Experiments[11]: A heart-lung preparation (Starling method) was perfused with the blood of a dog that had died of circulatory failure seven minutes after being immersed in hot water at 70 C.

Continuous records of the heart-lung preparation included aortic pressure (in millimeters of mercury by manometer), systemic minute output (total output less coronary flow), ventricular volume (Henderson cardiometer with a Kiese volume recorder) and oxygen consumption (spirometer).

The preparation had been used earlier for a study of the metabolic effects of alloxan, and the heart was failing spontaneously at the time at which the blood from the burned animal was introduced into the perfusing system (about three hours after the preparation had been isolated). Fifty cubic centimeters of the blood of the heated animal was injected into the venous return during a period of one minute. The minute output was about 130 cc. per minute at the time of the injection, and the injected blood reached the heart diluted two or three times with the original blood of the preparation.

Six minutes later 100 cc. of the blood of the burned animal was injected again in a period of one minute. This was diluted no more than once with the blood of the preparation.

Six minutes later the blood in the venous reservoir was removed and replaced with 200 cc. of blood of the burned animal. Following this, the last, addition the heart-lung preparation was being perfused almost entirely with the blood of the heated animal.

In none of the three trials was there any significant change in the aortic pressure, the minute output, the heart rate or the oxygen consumption. Although coronary flow was not recorded, any great increase in it such as might have been expected if the blood had contained as much as 0.5 mg. of histamine would have been recognized by an increase in the discrepancy between the stroke volume as recorded by the cardiometer and the stroke volume as calculated from minute output and heart rate. Such a change was not observed.

No deleterious effect resulted from perfusing the heart-lung preparation with the blood of the burned dog. Actually there was slight evidence

11. Dr. G. K. Moe, of the department of pharmacology of Harvard Medical School, gave assistance in the conduct of this experiment.

of a beneficial effect such as would be expected from the addition of any fresh blood after three hours of perfusion.

Relation of Systemic Hyperthermia to Survival.—There appeared to be a definite correlation between survival and the height to which the internal body temperature was raised. Attention has already been called to the fact that the maximum blood and tissue temperatures were reached within a few minutes after the conclusion of an exposure and that if an animal survived for more than thirty minutes the blood temperature fell and death either did not occur or was delayed for hours or days. With few exceptions, all of the animals that died soon after exposure were found to have marked elevation of rectal temperature. In the case of exposures of long duration and low intensity the rectal temperature taken at the time of death was only slightly lower than that of the blood of the right auricle. In animals that died within a few minutes after exposures of short duration and high intensity there was characteristically a difference of several degrees between rectal and blood temperature.

The correlation between the severity of systemic hyperthermia and the occurrence of early death is shown in figure 7. With one exception, all pigs that died during the early postexposure period were those in which the rectal or heart's blood temperature rose to 42.5 C. or higher. No pig whose rectal temperature rose to 44 C. or higher survived for more than a few minutes. Eleven of the 15 animals with rectal temperatures between 43 and 44 C. and 4 of the 13 with rectal temperatures between 42 and 43 C. died during the episode of hyperthermia.

Physiologic Changes.—Prior to exposure of several pigs to hot air, asbestos-insulated electrocardiographic leads were connected with the extremities, and a carotid cannula was introduced. The effect of the exposure on the rate and the amplitude of respiration, the pulse rate, the arterial blood pressure and the conduction system of the heart was observed.

Within a few seconds after exposure there was a sharp increase both in blood pressure and in rate of respiration. The respiratory rate continued to increase and remained rapid for some time after termination of the exposure. The blood pressure soon after the initial rise fell to or slightly below the preexposure level. In some animals the pressure was well maintained at that level until within a few minutes before death, whereas in others there was a gradual and progressive decline beginning immediately at the conclusion of the initial rise.

Electrocardiographic abnormalities [12] were observed in some animals soon after the beginning of exposure, whereas in others such changes

12. Dr. Howard Eder, of the department of medicine of Harvard Medical School, took and interpreted the electrocardiograms.

did not develop until well after the onset of circulatory failure. Abnormalities observed soon after the beginning of the exposure (within two or three minutes) included increase in rate, reduction in the voltage of the QRS complex and inversion of the T waves. Ventricular extrasystoles were noted and as the exposure was prolonged there were greater disturbances in rhythm. In such animals ventricular tachycardia developed, followed by fibrillation and death.

Although abnormalities were sometimes observed in the electrocardiogram before there was evidence of respiratory failure, the terminal

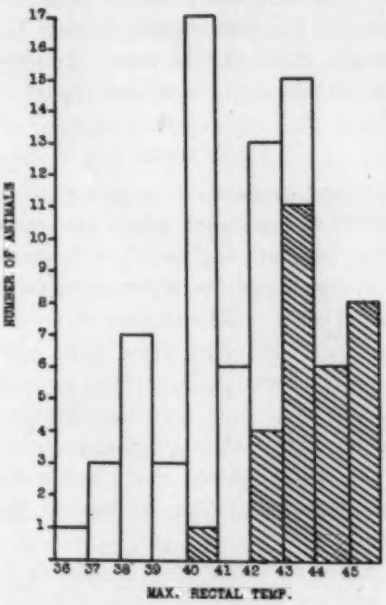

Fig. 7.—Distribution of animals according to the maximum thirty minute rise in rectal temperature following exposure to ambient and radiant heat. The initial temperatures were low because of pentobarbital sodium anesthesia. The open portions of the columns represent animals that survived; the shaded portions, animals that died during or within thirty minutes after exposure. It is apparent that there is a close correlation between systemic hyperthermia and death.

and agonal fall in blood pressure usually occurred about the same time that tachypnea gave way to intermittent periods of apnea.

The results of these experiments indicated that there are two types of hyperthermic circulatory failure, one central and the other peripheral. The former occurred in some animals as the result of brief exposures at high (over 200 C.) circumambient temperatures, whereas the latter occurred after long exposures at lower temperatures. It was obvious that further and more rigidly controlled physiologic experimentation

was required. Such studies were not feasible in the circumstances in which hot air experiments were conducted. See report VII of this series.[13]

Changes in Blood Potassium[14].—Samples of blood were withdrawn from 4 pigs by cardiac puncture before, during and after lethal exposures to hot air. It was found that the normal concentration of the potassium of the pigs' plasma was 3 to 5 milliequivalents per liter. The post-exposure concentrations were 7.3, 10.6, 17.4 and 19.6 milliequivalents per liter, respectively. The observation that cutaneous hyperthermia was capable of causing the plasma potassium to rise to as much as 17 milliequivalents per liter suggested acute potassium poisoning as a potential cause of death. Further investigation of the importance of the release of potassium as a cause of circulatory failure and death following exposure to heat will be presented in a later report (see articles VI and VII of this series[15]).

SUMMARY

In a series of pilot experiments in which gasoline was burned in a fireproof chamber with and without adequate ventilation, the changes that occurred in the oxygen, carbon dioxide and carbon monoxide contents and the temperature of the air were measured and the results of exposing animals to such conflagrations were observed. The most important information gained from these pilot experiments was that large animals exposed to such conflagrations may receive injuries that are almost immediately fatal and that the fatality is not necessarily contributed to by asphyxia, carbon monoxide poisoning or inhalation of flame or fumes. It was apparent that almost immediate death may result from systemic disturbances caused by the heat flowing through the surface of the body.

The time-temperature relationships responsible for varying degrees of cutaneous injury and for acute circulatory collapse and death incident to generalized cutaneous exposure to hot air and radiant heat were determined for the pig, and the probable effects of similar exposures of man have been discussed.

Source temperatures of approximately 90 C. could be tolerated for forty-five minutes without burning, whereas at 100 C. burning occurred in twelve minutes, and at 180 C. thirty seconds was the longest exposure that could be tolerated without irreversible cutaneous injury.

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13. Roos, A.; Weisiger, J. R., and Moritz, A. R.: Studies of Thermal Injury: VII. Physiological Mechanisms Responsible for Acute Circulatory Failure and Death During Cutaneous Exposure to Excessive Heat, J. Clin. Investigation, to be published.

14. Miss Regina McLean made the potassium analyses, which were based on the methods described by O. H. Lowry and A. B. Hastings (J. Biol. Chem **143**: 257, 1942).

15. McLean, Moritz and Roos.[8] Ross, Weisiger and Moritz.[13]

It was concluded that, although the temperature of the skin was considerably lower than that of the heat source in the hot air exposures, the relationships of time and tissue temperature that were responsible for cutaneous burning in these experiments were esssentially the same as those that prevailed in the previously reported hot water exposures.

At relatively low temperatures of heated air (under 120 C.) man, because of his ability to sweat, is undoubtedly less susceptible to injury than the pig. It is doubtful, however, that sweating provides a significant degree of protection at temperatures over 120 C., because at such levels the rate at which heat is transferred to the skin is considerably more rapid than the rate at which it can be dissipated by vaporization of sweat.

It should be borne in mind that the relationships of source temperature to production of injury that were established by these experiments apply only to unprotected skin and are not valid for exposures in which hair or clothing is interposed between the skin and the source of heat.

Excessive circumambient and circumradiant heat, though applied for periods as brief as thirty seconds, are capable of precipitating in pigs, dogs and goats physiologic disturbances of sufficient severity to cause death within a few minutes. The quantitative time-temperature characteristics of exposures which produce fatal systemic hyperthermia have been presented.

The severity of the physiologic disturbances that result when animals are exposed to excessive heat are frequently disproportionate to that of the cutaneous burning. Rapidly fatal systemic hyperthermia may result from long duration exposures at temperatures insufficient to cause cutaneous burning. Higher intensity exposures may cause extensive and severe cutaneous burning and yet be of too short duration to cause a significant rise in body temperature.

The severity of the immediate physiologic disturbances resulting from exposure to excessive heat appears to bear a direct relationship to the extent to which the body temperature is increased. Exposures that failed to cause the rectal temperature to rise above 42 C. rarely and those that caused it to rise as high as 44 C. invariably resulted in rapidly fatal circulatory failure. In the pig such a rise in body temperature may occur within a few minutes after cutaneous exposure to excessive heat and does not persist for more than a few hours after termination of the exposure. In animals that died within a few minutes after being exposed to excessively high environmental temperatures, the temperature of heart's blood was consistently higher than that recorded by a rectal thermometer, and the shorter the interval between the onset of exposure and death the greater was the difference.

There is no reason to believe that man and pig differ greatly in respect to the rate at which heat is transferred from the skin to the interior of the

body. If such is the case, man's susceptibility to development of rapidly fatal hyperthermia when exposed to environmental temperatures in excess of 120 C. is probably similar to that of the pig.

Apart from the cutaneous lesions, the pathologic changes observed at autopsy could not be construed as pathognomonic of thermal injury. Subendocardial ecchymoses were the most constant and striking internal abnormalities encountered in animals that died during or soon after exposure. Other characteristic postmortem findings included stasis of blood in the peripheral vascular bed, moderate dilatation of the right auricle and persistent fluidity of the blood. In the case of extensively burned animals there was intravascular hemolysis, and smears of blood disclosed fragmentation of erythrocytes. In animals that survived severe thermal exposures as long as twenty-four hours the urine was found to contain large amounts of hemoglobin and degenerative changes were frequently identified in the cells of renal tubules, the adrenal cortex and the liver.

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STUDIES OF THERMAL INJURY

V. The Predictability and the Significance of Thermally Induced Rate Processes Leading to Irreversible Epidermal Injury

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IN PREVIOUS STUDIES of the relationships that exist between time, temperature and the production of thermal cutaneous injury [1] a sufficient number of quantitative data were derived to establish the rate process inherent in the irreversible changes of epidermis injured by heat and to invite speculation as to the basic nature of these changes.

Some of the data were derived from an investigation [1a] in which a limited area of the skin was brought immediately to and maintained at a constant predetermined temperature and which included measurements (*a*) of the shortest time at which a constant predetermined cutaneous surface temperature produced transepidermal necrosis and (*b*) the longest time at which a constant predetermined surface temperature could be tolerated without the causation of irreversible transepidermal injury. Other data were derived from experiments [1b] in which the entire surface of the body was exposed to hot air and radiant heat, with observations of (*c*) the shortest time at which circumambient and circumradiant heat of measured intensity caused transepidermal necrosis. These three threshold values for epidermal injury as related to time and temperature will be hereafter referred to as thresholds A, B and C, respectively.

Because the effect which temperature has on the physical or the chemical state of matter is dependent on time, the production of the effect is known as a rate process, and it is the purpose of this communi-

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1. (*a*) Moritz, A. R., and Henriques, F. C., Jr.: Studies of Thermal Injury: II. The Relative Importance of Time and Surface Temperature in the Causation of Cutaneous Burns, Am. J. Path., to be published. (*b*) Moritz, A. R.; Henriques, F. C., Jr; Dutra, F. R., and Weisiger, J. R.: Arch. Path., this issue, p. 466.

cation to show that a general expression which applies to all rate processes enables the development of a mathematical equation which quantitatively predicts these time-temperature thresholds of epidermal injury—and to evaluate so far as possible the theoretic significance of this equation.

The development and the evaluation of this equation presuppose accurate knowledge of three parameters: duration of thermal exposure, degree of epidermal injury as judged histologically and temperature attained at the basal epidermal layer during the entire hyperthermic episode—this cell layer being specifically selected, since increases of the temperature of these cells are of fundamental import in the production of cutaneous necrosis. The first two parameters are directly determinable, while the last can be accurately evaluated from the fundamental laws of the transfer of heat and the type of thermal exposure.

The theory which enables the evaluation of the time dependence of the temperature of the basal epidermal layer is considered in detail in number I of "Studies of Thermal Injury" [2] and is based on the excellent premise that the ratio of total tissue thickness to epidermal thickness (about 80 microns) is infinite rather than a large finite quantity—"infinite body picture."[2]

This theory shows that under the experimental conditions applicable to thresholds A and B the time-temperature relationship at the basal epidermal layer can be expressed by the following equations:

$$\frac{T_t - T_i}{T_s - T_i} = \frac{2}{\sqrt{\pi}} \int_0^{\gamma\sqrt{t}} e^{-x^2}\,dx \qquad (1)$$

where

$$\gamma = \frac{L}{2\sqrt{K/C_p\rho}} = 0.15 \qquad (2)$$

where

T_t = the temperature of the basal epidermal cells at the time (t) in seconds

T_s and T_o = the temperatures of the cutaneous surface during and previous to the thermal exposure, respectively

L, K, C_p and ρ = the thickness, the thermal conductivity, the heat capacity and the density, respectively, of the epidermis

By substituting the experimental values [3] of L, K, C_p into equation 2, a value of 0.15 can be computed for γ if an epidermal density of 0.8 Gm. per cubic centimeter is assumed.

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2. Henriques, F. C., Jr., and Moritz, A. R.: Studies of Thermal Injury: I. The Conduction of Heat to and Through the Skin and the Temperatures Attained Therein; a Theoretical and an Experimental Study, Am. J. Path., to be published.

The integral (equation 1) is equal to $\pi/2$ and zero when time (t) is zero and infinite, respectively, and thus after a certain interval of time, equation 1 reduces to

$$\bar{\tau} = \tau \qquad (3)$$

which states that the temperature of the basal epidermal layer ultimately becomes identical with that of the cutaneous surface. Actually there will always be a small but finite temperature gradient between the surface and the basal cell layer. Fortunately, this small difference of temperature need not be considered, since these threshold A and B data are such that for all cutaneous surface temperatures above 50 C. the duration of exposure is too short to allow the epidermis to reach heat saturation and for all temperatures below 50 C. the difference (< 0.2 C.) between the steady state temperature of the basal epidermal layer [2] and the temperature of the cutaneous surface is trivial.[2] Hence equation 1 will accurately evaluate the time-temperature relationship over the range of the experimental exposure times applicable to thresholds A and B.

An expression similar to but more complicated than equation 1 has been derived for the experimental condition pertaining to threshold C. The temperatures of the basal epidermal layer have been computed and tabulated for circumambient temperatures of 80 C., 100 C., 125 C., 150 C. and 175 C., respectively (equation 6 and table 6 of number I in this series),[2] and these data are directly applicable to the ensuing discussion.

PREDICTABILITY OF THERMALLY INDUCED EPIDERMAL INJURY

If the reaction leading to the thermal death of epithelium conforms to that of most chemical and physical rate processes,[3] it should quantitatively bear out the following equation:

$$\frac{d\Omega}{dt} = P e^{-\Delta E/R(T_t+273)} \qquad (4)$$

where

$d\Omega/dt$ = the rate at which Ω, an arbitrary function of epidermal injury as determined by histologic examination, is produced

T_t = the temperature in degrees Centigrade at the basal epidermal layer at the time (t) in seconds

R = the gas constant. It is equal to 2 calories per degree Centigrade per mol

P and ΔE = constants evaluated from the experimental data.

Equation 4 can also be expressed as an integral equation, namely

$$\Omega = P \int_0^t e^{-\Delta E/R(T_t+273)} dt \qquad (5)$$

where if T_t, the time dependence of the temperature of the basal layer

3. Glasstone, S.; Laidler, K. J., and Eyring, H.: The Theory of Rate Processes, New York, McGraw-Hill Book Company, Inc., 1941.

of epidermis, can be considered invariant during heat exposure, equation 5 can be integrated to equation 6,

$$\Omega = P t e^{-\Delta E/R(T+273)} \qquad (6)$$

where T is the steady state temperature at the basal layer. This enables one to make the numerical evaluation of P and ΔE from a limited set of experimental observations.

Thresholds A and B.—An examination of data pertaining to threshold A and the epidermal time-temperature data as given by equation 1 (see table 5 of study I in this series [2]) shows that for cutaneous surface temperatures less than 50 C. the hyperthermic episode is sufficiently long to enable the use of equation 6; furthermore, the cutaneous surface temperature can be substituted for the steady state temperature of the basal epidermal cells (equation 3).

Thus, by using equation 6 in this temperature range, it is possible to evaluate numerically P and ΔE by standard graphic procedures from the threshold A data, and obtain

$$\Delta E = 150{,}000 \text{ CALORIES PER MOL} \qquad (7)$$

and

$$P = 3.1 \times 10^{98} \text{ SEC}^{-1} \qquad (8)$$

This value of P depends on the arbitrary choice of the value of unity for Ω. Thus, when threshold A, complete epidermal necrosis, is reached,

$$\Omega \equiv 1 \qquad (9)$$

By again making use of equation 6, a similar analysis can be made of the threshold B data. Since these data are not so complete as those used in the foregoing discussion, it is best to use the same numerical values given by equations 7 and 8 for ΔE and P, and solve for the numerical value of Ω.

These data are best represented by

$$\Omega \equiv 0.53 \qquad (10)$$

when the maximum exposure time which can be tolerated without the occurrence of epidermal necrosis is reached.

Although the values given by equations 7 to 10 for P, ΔE and Ω were obtained by means of equation 6 and a restricted portion of the experimental data, they should enable one to compute these two thresholds over the entire experimental temperature range through equation 5 so long as T_t is known.

It is possible to ascertain T_t, the time dependence of the temperature of the basal epidermal layer, by means of equation 1, where its evaluation depends on two parameters, T_o and γ. An examination of this equation shows that T_t is quite insensitive to variations in T_o, the original cutaneous surface temperature, and 35 C. will be the value used.[2]

In view of the uncertainties [2] which enter into the direct experimental evaluation of γ, its numerical value, 0.15, given under equation 2 was ascertained by obtaining the best fit to the complete threshold A data.

A consideration of equations 1 and 5, together with the requisite numerical values given by equations 2, 7 and 8, shows that all of these experimental data are completely described by the following equations:

$$\Omega = 3.1 \times 10^{98} \int_0^t e^{-75,000/(T_t+273)} dt \qquad (11)$$

where

$$T_t = T_s - (T_s - 35)\left[\frac{2}{\sqrt{\pi}} \int_0^{0.15/\sqrt{t}} e^{-y^2} dy\right] \qquad (12)$$

Ω = the degree of injury to be expected

T_s = the surface temperature of the skin during heat exposure

T_t = the temperature of the basal epidermal layer after the time (t) in seconds has elapsed.

The numerical values of the integral of equation 12 as a function of the limits (zero and $0.15/\sqrt{t}$) are tabulated.[4]

For $\Omega > 0.5$ and $T_s < 50$ C. the time dependence of T_t can be ignored and T_t put equal to T_s; equation 11 can then be integrated and takes the form of equation 6, which greatly facilitates the computation of Ω. With T_s higher than 50 C. and $\Omega \leqq 1$ the time dependence of T_t cannot be neglected, and the evaluation of Ω requires one of the standard methods of numerical integration.[5]

This numerical determination of Ω from the two experimental parameters t and T_s enables the prediction of the degree of epidermal injury, since an $\Omega \leqq 0.53$ results in a time-temperature relationship that can be tolerated without the occurrence of irreversible epidermal injury (threshold B), and $\Omega \geqq 1.0$ results in a time-temperature relationship which produces complete epidermal necrosis (threshold A).

The success with which equations 11 and 12 predict these time-temperature relationships is shown in table 1.

It can be seen that in general the experimental threshold data agree excellently with those predicted, and thus the applicability of equation 4 which has been tacitly assumed throughout this section is demonstrated. In the 4 cases in which there is appreciable variance between experimental data and predictions, either the experimental data are insufficient or the duration of heat exposure was too short to preclude considerable experimental error.[1a] Thus equations 11 and 12 probably give a more accurate estimation of thresholds A and B than the published experimental curves (fig. 4).[1a]

4. Pierce, B. O.: A Short Table of Integrals, Boston, Ginn and Company, 1929.

5. Ford, L. R.: Differential Equations, New York, McGraw-Hill Book Company, Inc., 1933.

The numerical computations resulting from equation 6 are also included for comparative purposes. For reasons previously stated, there is no appreciable difference between this equation and equation 11 for all cutaneous surface temperatures below 50 C. under these specific experimental conditions. Equation 6 corresponds to an experimental condition in which the basal epidermal layer is brought immediately to and maintained at a constant temperature. If this were feasible at 70 C., complete epidermal necrosis would result in three ten-thousandths of a

TABLE 1.—*Predicted Time-Temperature Thresholds of Epidermal Injury Compared with Those Experimentally Observed (The Data are Valid Only for a Thermal Exposure in Which the Surface of the Skin is Brought Immediately to and Maintained at a Constant Predetermined Temperature)*

Minimum Exposure in Seconds Resulting in Complete Transepidermal Necrosis (Threshold A) $\Omega \equiv 1$			Cutaneous Surface Exposure Temperature in Degrees Centigrade	Maximum Exposure in Seconds Resulting in Reversible Epidermal Injury (Threshold B) $\Omega \equiv 0.53$		
Equation 6*	Equations 11 and 12	Experimental Threshold A†		Experimental Threshold B†	Equations 11 and 12	Equation 6*
23,000	23,000	25,000	44	18,000§	12,000	12,000
11,000	11,000	11,000	45	7,200§	5,900	5,800
5,100	5,200	5,000	46	3,000	2,800	2,700
2,400	2,500	2,400	47	1,300	1,380	1,300
1,100	1,200	1,100	48	560	650	600
580	630	570	49	260	340	310
270	325	300	50	130	165	140
130	165	160	51	75	90	68
65	91	90	52	44	52	35
16	31	35	54	18	19	8
4.4	12	16	56	8.3	8.1	2.3
0.25	3.0	5	60	2.6§	2.3	0.13
0.009	1.0	2§	65	1.0§	0.7	0.005
0.0003	0.5	1§	70	....	0.4	0.0002

* Above 50 C. equation 6 has no experimental significance.

† The data are reported in number II of "Studies of Thermal Injury" (see fig. 4).[1a]

§ The experimental value is uncertain.

second. The two thousand fold difference between this value and the five-tenths second predicted by equations 11 and 12 indicates the extreme importance of the thermal capacity and conductance of the skin during the early period of heat exposure.

These tabulated values resulting from the solution of equation 11 are, of course, valid only under specific experimental conditions, namely, when the cutaneous surface temperature is immediately brought to and maintained at a constant value during the entire heat exposure.

However, equation 11 should accurately predict the degree of epidermal injury to be expected from all conceivable kinds of heat exposures so long as the temperature dependence of the cutaneous surface temperature as a function of time can be evaluated since the time-temperature relationship at the basal epidermal layer can always

be determined by means of the "infinite body" heat theory [6] implicit in equation 12. The validity of this generalization is demonstrated in the next section, where the experimental threshold C data are compared with the values predicted by means of equation 11.

Threshold C.—In order to presage these data from equation 11, T_t, the time dependence of the temperature of the basal epidermal layer, must be evaluated under conditions in which the entire cutaneous surface is exposed to a constant source of circumambient and circumradiant heat. Values of T_t as a function of the time of thermal exposure under the requisite experimental conditions [1b] have been computed and are tabulated in number I (table 6) of this series.[2]

The predicted threshold data, which were computed by substituting these basal temperatures into equation 11 and then integrating graphically until Ω equaled 1, are compared with those determined experimentally in table 2; comparisons were made at five air temperatures ranging from 80 to 175 C., which bounded the entire experimental range.

TABLE 2.—*A Comparison of the Threshold C Data with Those Predicted (The Data are Valid Only for Circumambient and Circumradiant Heat Exposures.)*

Air Temperature in Degrees Centigrade	Minimum Exposure Time in Seconds Resulting in Complete Transepidermal Necrosis	
	Experimental Threshold C	Threshold C Predicted by Equation 11
80	700	900
100	300	400
125	200	150
150	85	80
175	35	45

In view of the limited experimental data on which threshold C is based [1b] and the uncertainty of the temperatures of the basal epidermal layer as computed under these experimental conditions,[2] the concordance of the experimental and the predicted values is excellent; thus, considerable confidence can be placed in the statements of the last paragraph in the previous subsection.

THE NATURE OF THE CHANGES INDUCED IN EPIDERMIS BY HYPERTHERMIA

In the previous section it was shown that the degree of epidermal injury resulting from a thermal exposure can be quantitatively predicted by the standard expression for a rate process, specifically equation 4.

6. Carslaw, H. S.: Mathematical Theory of Conduction of Heat in Solids, New York, The Macmillan Company, 1921. Henriques and Moritz.[2]

In this equation there appear two empiric and experimentally determinable constants, namely, P and ΔE; any theoretic consideration of the cause of thermally induced transepidermal necrosis should take into account at least qualitatively the numerical values of these quantities. Aside from certain general conclusions regarding the entropy of the over-all process,[3] little specific information can be obtained from the numerical value of P, since this constant is intimately connected with the as yet unknown detailed physical and chemical properties and functions of the epidermal constituents. This is not the case, however, with ΔE, and thus, before proceeding further, a brief general consideration of the nature of ΔE, the activation energy in calories per mol, is in order.

Thermal Injury and Energy of Activation.[3]—In general, the kinetics of any given physical and/or chemical process depends on the total energy content of the constituents involved. If this energy content is less than a certain critical value, known as the activation energy, the process cannot take place, and if the energy content is greater than this critical value, the process may take place; thus, the rate of the process will be proportional to the fraction of these constituents which, collectively considered, possess an energy content at least equal to the activation energy. This fraction is deduced from the Maxwell-Boltzman energy distribution law, which states that [3]

$$f = e^{-\Delta E/R\,(T+273)} \qquad (13)$$

where f is this fraction. The remaining symbols have been previously defined. Equation 13 determines only the temperature coefficient of a rate process, since, as shown by equation 4, the rate of a process is also proportional to one other factor that is essentially nondependent on temperature, namely, P.

Thus, the rate of any conceivable process that may result in cell death, whatever it may be, depends on a critical energy content for the participants. The fraction of the participants, collectively considered, having this energy is determined by the activation energy and the temperature (equation 13); however, the availability of this fraction is requisite but not in itself sufficient to allow the process to proceed.

An inspection of equation 13 shows that the temperature coefficient of any kinetic process is a strong function of the activation energy; for example, in the neighborhood of 50 C. the rate of a process with an activation of 1, 10 or 100 kilocalories per mol will be altered by about 0.4, 7 or 70 per cent, respectively, per unit change of temperature in degrees centigrade.

The kinetics of a considerable number of physical and chemical phenomena has been studied in detail, and it is possible to classify all rate processes, and hence, in particular, those mechanisms which may

be of considerable importance in the general consideration of thermal injury, according to the order of magnitude of their activation energy.

During the past fifty years, numerous theories [7] have been proposed to explain injuries thermally induced in living organisms. Before applying the aforementioned criteria to the mechanisms involved in these theories, it is necessary to characterize briefly the attributes of a living cell.[8]

The living cell appears to consist of a semirigid, relatively nonsoluble framework (e. g., nucleus, nuclear wall and cell wall) that is primarily protein in nature. This aggregate is bathed in an aqueous intracellular fluid which contains both particulate (e. g., micelles) and soluble constituents, which range from simple ions to cytoplasmic proteins of extraordinary complexity. Aside from having certain purely physical attributes (e. g., permeability, contractibility, elasticity, cohesiveness, rigidity and tensile strength), this protoplasmic entity respires, excretes, synthesizes all imaginable types of molecules, utilizes and liberates energy, and reproduces in a manner that perpetuates its own kind. This exceedingly complex metabolic activity is apparently both catalyzed and precisely controlled by a multiplicity of enzymatic proteins and functionally allied molecules which contribute to both the structural framework and the cytoplasmic fluid.

In view of this complex picture, no theory of thermal injury can be considered tenable unless it takes into account these completely integrated and precisely balanced phenomena which, taken as a whole, comprise cell life. Unfortunately, as yet, knowledge of these phenomena is meager and is limited to isolated observations on living protoplasm (e. g., cell respiration, mitosis, diffusion of a few substances through cell walls) and to certain chemical and physical properties and functions of a few of the molecules that can be extracted in a presumably unaltered state from dead cell brei.

Nevertheless, even on the basis of this limited information, it is interesting to speculate in regard to the general kinds of mechanisms that may be of importance in explaining the quantitative time-temperature relationship that results in irreversible epidermal injury as judged morphologically. These data [1] concerning injury showed that episodes of transepidermal injury can be quantitatively predicted by a rate equation with an activation energy of 150 kilocalories per mol (equation 7) over the entire experimental temperature range (cutaneous surface temperatures from 44 to 70 C. and circumambient temperatures from 80 to 175 C.).

7. Belehradek, J.: Temperature and Living Matter, Berlin, Verlag von Gebrüder Borntraeger, 1935.

8. Cattell, J.: Biological Symposia, Lancaster, Pa., Jaques Cattell Press, 1943, vol. 10.

The theories[7] that have been advanced to explain thermal injury may be classified into three general groups:

1. Thermal alterations in proteins.

In view of the many varied functions of the proteins that contribute to the maintenance of normal cell life, it is obvious that even minor thermally induced alterations of these molecules may result in profound irreversible injuries. For example, these thermal protein changes could produce increased permeability of the nuclear and/or the cell wall, structural alterations in the nucleus itself, disintegration of the protein mitochondria present in the cytoplasm and inactivation of enzymes.

Many quantitative studies[9] have been made on the effects of temperature on proteins, and alterations that proceed at a measurable rate between 0 and 100 C. with activation energies in excess of 50 kilocalories per mol are not unusual. The heat inactivation of invertase (ΔE — 110 kilocalories at p_H 4 and ΔE — 52 kilocalories at p_H 5.7) and of peroxidase (ΔE — 189 kilocalories) and the heat denaturation of egg albumin (ΔE — 132 kilocalories at p_H 5) and of hemoglobin (ΔE — 76 kilocalories at p_H 6.8) are a few of the many examples.

Thus, the morphologic observations of protein dissolution and/or coagulation on which the quantitative judgment of transepidermal necrosis is based may well be directly due to the thermal alterations of as yet unknown proteins present in epidermal cells.

2. Other possible alterations in metabolic processes.

Since temperature affects to a greater or a lesser degree the kinetics and the thermodynamics of all chemical and physical phenomena, heat may cause alterations in metabolism irrespective of its effect on proteins. For example, the entire metabolic equilibrium may be upset if the concentrations of some of the individual constituents have changed as a result of temperature-induced variations in the rates of diffusion, formation and degradation of the chemical reactants comprised in the process; in fact, owing to this abnormal functioning, certain metabolites normally present may completely disappear and/or others abnormal and toxic in character may arise. There can be no doubt that these phenomena take place and that they may cause cell death.

Many of these metabolic reactions,[9a, b] both enzyme and nonenzyme catalyzed, have been studied as in vitro processes, and activation energies usually between 10 and 20 kilocalories per mol are generally found. In certain instances the activation energies are less than 10 kilocalories per mol, but none have been found to exceed 50 kilocalories.

To date there is no experimental evidence that these types of reactions can lead to a temperature coefficient for thermal injury which corresponds to that found experimentally for transepidermal necrosis.

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9. (*a*) Sumner, J. B., and Somers, G. F.: Chemistry and Methods of Enzymes, New York, Academic Press, Inc., 1943. (*b*) Bull, H. B.: Physical Biochemistry, New York, John Wiley & Sons, Inc., 1943. (*c*) Glasstone and others.[3]

3. Nonprotein induced alterations in the physical characteristics of cells.

Into this group are placed all physical phenomena that are characteristic of protoplasm but are not primarily affected by the thermal alterations of proteins contained therein. For example, diffusion of metabolites through a cell wall that has not undergone chemical alteration is a member of this class, while changes in diffusion rates that are the result of an increased permeability of cell wall due to degradation of the structural protein are specifically excluded, since this phenomenon is classified under group 1.

All of the biophysical rate processes that have been studied, such as diffusion through liquids and membranes, changes in viscosity, rigidity or tensile strength, and liquefaction, possess activation energies that are usually less than 5 kilocalories per mol and never in excess of 15 kilocalories per mol.

Although these types of mechanisms are undoubtedly potentially capable of causing cell death, they are not the instigators of the morphologic changes that are observed in irreversible epidermal injury.

Since many fatlike substances are known to melt around 45 C., the liquefaction of such substances has received some consideration as a potential instigator of thermal injury.[7] From a kinetic standpoint the rate of melting is a physical process with activation energy essentially zero. This theory would predict that there would be a sharp temperature threshold for injury and that then the injury rate would become nearly a linear function of the increment in temperature above the threshold value. Hence, while liquefaction might account for the quantitative epidermal thermal relationships [1a] at cutaneous surface temperatures between 45 and 48 C., there would be extreme variance with the experimental data at the higher cutaneous temperatures. The extent to which thermal liquefaction of lipoid substances may contribute to cell death in tissues other than the epidermis was not investigated.

In view of the foregoing discussion it can be concluded that the only biokinetic phenomena known to date that can account for epidermal cell death are the thermally induced changes in protein structure which have an activation energy in the neighborhood of 150 kilocalories per mol.[10] This in no way excludes the injury propensity of the innumera-

10. Crozier and his co-workers have used the concept of activation energy to interpret many life processes by means of some master reaction. This interpretation has been criticized by numerous investigators (Bull.[9b] Heilbrunn, L. V.: An Outline of General Physiology, Philadelphia, W. B. Saunders Company, 1943), since it is mathematically demonstrable that the constancy or inconstancy of the activation energy is neither a necessary nor a sufficient condition to prove the respective existence or nonexistence of a specific master reaction. In Crozier's investigations, the activation energies found were usually in the neighborhood of

(Footnote continued on next page)

ble mechanisms implied earlier, but merely states that all quantitative studies made in this investigation indicate that the morphologic changes observed in the thermally altered epidermal tissue can be ascribed to these degradations of proteins.

As to the number and the kinds of proteins involved, the specific nature of thermally induced reactions and the individual rate of each protein alteration, nothing can be stated. Further, it is probable that at any given hyperthermic level any one of theses numerous protein alterations is potentially capable of producing cell death.

Thermal Injury and Entropy and Free Energy of Activation.—With no intention whatsoever of inferring that the thermal effects on living protoplasm can be ascribed to the alteration of any single protein, it is of value to make for the moment this extreme oversimplification in order to interpret the significance of the numerical value of P in the empiric rate equation 4, which predicts completely the thresholds of transepidermal necrosis.

In vitro studies on both enzymatic and nonenzymatic proteins have shown that the rate of thermally induced changes is first order, and the quantity of degraded protein is given by [8]

$$\ln \frac{C_0}{C_t} = \frac{k(T+273)}{h} t \left[e^{\Delta S/R}\right]\left[e^{-\Delta E/R(T+273)}\right] \qquad (14)$$

where

C_0 = the amount of protein originally present

C_t = the amount of unaltered protein present at the time (t) in seconds

K(1.37 × 10^{-16} ergs per degree), h(6.55 × 10^{-27} ergs-seconds) and R(2 calories per degree per mol) = the Boltzman, Plank and Gas constants, respectively

T = the temperature in degrees centigrade

ΔS and ΔE = the entropy and the activation energy of the rate process, respectively.

Comparing this equation with equation 6 and remembering that $\Omega \equiv 1$ for the production of transepidermal necrosis, one finds in the neighborhood of 50 C. the following numerical relationship for ΔS in calories per degree per mol.

$$\Delta S = 398 + 2 \ln (\ln C_0/C_t) \qquad (15)$$

where C_0/C_t is the reciprocal of that fraction of the original protein present which is still unaltered at the onset of transepidermal necrosis.

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10 to 20 kilocalories, and since the great majority of biologic reactions are in this range, these criticisms are well justified. However, it is also mathematically demonstrable that no conceivable combinations of a series of reactions with activation energies within a certain bound can produce an over-all kinetic process with an activation energy out of this bound (e. g., no combination of reactions, for which the activation energies are greater than 10 kilocalories but less than 20 kilocalories; can lead to any over-all phenomena with an activation energy less than 10 kilocalories or greater than 20 kilocalories). Thus, the interpretation in the text is valid.

This equation is extraordinarily insensitive to the ratio C_o/C_t. Thus, the variation of this ratio from 100 (99 per cent protein alteration requisite for injury) to 1.01 (1 per cent alteration required), changes ΔS from 401 to 389. In view of this fact the analysis can at once be generalized to include all of the simultaneous inactivations and denaturations of the numerous protoplasmic proteins which are thermally induced and proceed with an activation energy in the neighborhood of 150 kilocalories per mol.

Thus, for the combined effect of these processes one finds that

$$\Delta S \rightleftharpoons 395 \text{ ENTROPY UNITS} \qquad (16)$$

The free energy of activation, ΔF, can be computed from this entropy of activation and the experimental activation energy (150 kilocalories per mol, equation 10) from the following thermodynamic equation.[8]

$$\Delta F = \Delta E - (T + 273)\Delta S \qquad (17)$$

And in the neighborhood of 50 C. one obtains

$$\Delta F \rightleftharpoons 22 \text{ KILOCALORIES PER MOL} \qquad (18)$$

High energies of activation occurring together with large increases in entropy, which lead to free energies of activation of from 20 to 30 kilocalories in the neighborhood of 50 C., are a unique characteristic of all rates of heat denaturation of proteins and heat inactivation of enzymes which have been quantitatively studied in vitro.[8]

Certainly it is more than coincidence that the numerical constants of an experimental equation that quantitatively predicts thermally induced epidermal necrosis are in complete accord with the known temperature dependence of the in vitro alterations in protein structure, and thus one can place considerable confidence in the statement that thermally induced injury of living epidermal protoplasm is primarily due to changes in some of the nuclear and cytoplasmic proteins which have activation energies for thermal degradation in the neighborhood of 150,000 calories per mol.

LATENT THERMAL INJURY

In articles II and III of "Studies of Thermal Injury" the existence of latent or morphologically unrecognizable epidermal cellular injury following certain apparently harmless thermal exposures was proved by repeated application of prethreshold exposures. Furthermore, the time required for recovery after these exposures producing latent injury became longer the nearer the changes approached microscopic visibility.[11]

11. Moritz, A. R.: Studies of Thermal Injury: III. The Pathology and Pathogenesis of Cutaneous Burns; an Experimental Study, Am. J. Path., to be published. Moritz and Henriques.[1a]

The concept of an unknown but definite fraction of certain of the cellular proteins that must be thermally altered before morphologically recognizable injury will result is in agreement with these experimental data.

During a thermal exposure that results in latent injury a noncritical fraction of these proteins are altered. At the termination of the exposure the epidermis rapidly approaches normal temperature,[2] and at least partial cell function is resumed. Thus, during the period of recovery the thermally altered proteins are replenished to a degree which depends in part on the length of the period of recovery and in part on the duration of the thermal exposure which produced unrecognizable injury.

SUMMARY

The experimental time-temperature relationships which determine the thresholds of epidermal injury (studies II and IV of "Studies of Thermal Injury") are subjected to mathematical analyses. Two types of thermal exposure are considered: those in which the cutaneous surface is immediately brought to and maintained at a constant temperature, and those in which the skin is exposed to a constant source of circumambient and circumradiant heat.

It is demonstrated that all of these data are quantitatively predicted by the following equation:

$$\Omega = 3.1 \times 10^{98} \int_0^t e^{-75{,}000/(T_t + 273)}\, dt \qquad (II)$$

where T_t is the temperature in degrees centigrade of the basal epidermal layer at the time (t) in seconds. The values of T_t during the hyperthermic episode depend on the type of thermal exposure and are evaluated by means of equations developed in study I of "Studies of Thermal Injury."

All values of Ω less than or equal to 0.53 result in a time-temperature relationship that can be tolerated without the occurrence of irreversible epidermal injury; all those equal to or greater than 1 result in a time-temperature relationship which produces transepidermal necrosis.

A theoretic treatment of this equation shows that, of all the physical and chemical processes known to date, thermally induced epidermal injury must be due primarily to thermal changes of those proteins and enzymes whose rate of alteration corresponds to an activation energy of at least 150 kilocalories per mol and an entropy change of about 395 units. This is consistent with the known existence of a reversible phase of latent epidermal injury.

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NEPHROTOXIC ACTION OF *DL*-SERINE IN THE RAT

I. The Localization of the Renal Damage, the Phosphatase Activity and the Influence of Age, Sex, Time and Dose

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IN THE course of experiments dealing with the influence of dietary factors on the composition of tissue phospholipids, Fishman and Artom [1] observed a damaging effect of *dl*-serine (alpha-amino beta-hydroxypropionic acid) on the kidneys of young rats. They found that 50 to 100 mg. of *dl*-serine given by stomach tube or injected parenterally was injurious, while even the double amount was not toxic when added to the food.[2] The toxicity of *dl*-serine was markedly influenced by the diet given. Rats on an adequate stock diet had a lower mortality than those on a diet of 10 to 30 per cent casein, as the source of protein, and deficient in B vitamins. The renal anatomic changes were studied by Morehead, Fishman and Artom.[3] Twenty-four hours after the initial administration of *dl*-serine they observed acute necrosis at the junction of the renal cortex and medulla. Even when the administration of *dl*-serine was continued for fourteen days resorption of the necrotic tissue took place and almost complete repair occurred in rats supplied a complete diet. In rats which were fed a diet deficient in B vitamins and low in protein the renal damage was more severe. Calcium deposits were marked after several days of the administration of *dl*-serine, and healing took place with considerable scar formation and resulting hypertrophy of the remaining undamaged tubules. While the addition of pure B vitamins to the experimental diets considerably reduced the mortality and the severity of the clinical symptoms,[4] it did not influence the extent of the renal lesion.[5] A

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1. Fishman, W. H., and Artom, C.: J. Biol. Chem. **145**:345, 1942.
2. Artom, C., and Fishman, W. H.: Proc. Soc. Exper. Biol. & Med. **57**:239, 1944.
3. Morehead, R. P.; Fishman, W. H., and Artom, C.: Am. J. Path. **21**: 803, 1945.
4. Fishman, W. H., and Artom, C.: Proc. Soc. Exper. Biol. & Med. **57**:241, 1944.
5. Morehead, R. P.; Fishman, W. H., and Artom, C.: Am. J. Path. **22**:385, 1946.

comparison of the relative toxicity of *l*-serine and of *dl*-serine led Artom, Fishman and Morehead [6] to the conclusion that the toxic action was due to the unnatural *d*-isomer, since *l*-serine had no injurious effect. In young guinea pigs and rabbits, as well as in mature mice, comparable lesions of the kidneys were not found.[7]

In the present study, various factors concerning the nephrotoxic action of *dl*-serine in the rat were observed. The influence of age and sex was examined, as well as the minimal toxic dose and the time after which the first evidence of renal damage could be ascertained under the microscope. An attempt was also made by several methods to determine the exact location of the tubular damage. In addition, the influence of acute *dl*-serine poisoning on renal phosphatase activity was studied.

MATERIAL AND METHODS

Albino rats of the Wistar strain were used. Except in the experiments dealing with the influence of sex and age, they were male animals weighing approximately 100 Gm. They were fed either "Rockland rat diet complete" (Arcady Farms, Milling Co., Chicago) or, for a period of seven days, a protein-deficient, vitamin B complex–free diet of a composition similar to that of the diet 1 used by Fishman and Artom.[1] This diet consisted of vitamin test casein (General Biochemicals) 10 per cent, dextrin 39 per cent, sucrose 37 per cent, hydrogenated cottonseed oil ("crisco") 5 per cent, cod liver oil 5 per cent and salt mixture no. 2 U. S. P. XII 4 per cent. Each animal received only a single dose of *dl*-serine (Merck), either by stomach tube or by intraperitoneal injection. In a few instances the amino acid was injected intravenously into a tail vein. Unless otherwise stated, the animals were killed after twenty-four hours. Either 50 or 100 mg. of *dl*-serine dissolved in 3 cc. of water was given regularly except in those experiments in which the influence of the dose on the renal damage was examined. Tissues were fixed in 8 per cent solution of formaldehyde U. S. P. and stained with hematoxylin and eosin. For the demonstration of alkaline phosphatase activity, acetone-fixed tissues were stained according to Gomori's [8] method as modified by Kabat and Furth.[9] The slides were incubated for two to twelve hours in the buffer-phosphate mixture. No counterstain was used.

An attempt was made to localize the renal damage exactly by vital staining and by isolation of single renal units. Vital staining of the proximal convoluted tubules took place after 1 cc. of lithium-carmine solution had been injected subcutaneously on five consecutive days. Simultaneously with the last injection of this solution, *dl*-serine was given intraperitoneally, and the animal was killed twenty-four hours later. Sections of tissue fixed in solution of formaldehyde U. S. P. and acetone were slightly counterstained with Harris' hematoxylin. The isolation of single renal tubules was performed by Dr. Jean Oliver in the department of pathol-

6. Artom, C.; Fishman, W. H., and Morehead, R. P.: Proc. Soc. Exper. Biol. & Med. **60**:284, 1945.

7. Morehead, R. P.; Poe, W. D.; Williams, J. O., and Lazenby, M. E.: Am. J. Path. **22**:658, 1946.

8. Gomori, G.: Proc. Soc. Exper. Biol. & Med. **42**:23, 1939.

9. Kabat, E. A., and Furth, J.: Am. J. Path. **17**:303, 1941.

ogy of the Long Island College of Medicine, Brooklyn. This method consists essentially in isolating single nephrons from formaldehyde-fixed tissue that had been treated previously with strong hydrochloric acid and staining them in toto with a modified iron-hematoxylin procedure, ferric chloride being used as mordant.[10]

RESULTS

Localization of the Renal Injury.—Eighteen rats fed the complete, and 13 rats fed the synthetic diet all showed renal lesions after the administration of 100 mg. of *dl*-serine. On gross examination a small, irregularly shaped strip of grayish discoloration at the innermost portion of the cortex indicated the necrotic area. Microscopically, the changes agreed closely with those described by Morehead, Fishman and Artom.[11] Acute renal necrosis occurred at the junction of cortex and medulla twenty-four hours after the administration of *dl*-serine. The epithelium of many tubules in this area was almost completely necrotic (fig. 1).

The changes observed varied somewhat in severity in different fields. Casts composed of eosinophilic granular material were frequent, located at times in the convoluted, but mostly in the collecting tubules. Not all the tubules at the corticomedullary junction, however, were necrotic. Even where the damage was most extensive, occasional tubules were spared. In animals restricted to the synthetic diet the renal damage induced by *dl*-serine was more extensive. In some fields the necrosis extended into the more outlying portions of the cortex. The glomeruli were not affected.

The areas of necrosis were located predominantly in the outer strip of the outer zone of the medulla, according to the nomenclature of Peter,[12] which corresponds to the subcortical zone of Heidenhain.[13]

According to Heidenhain, the cortex can be divided into three zones. The narrow outermost zone (cortex corticis) contains no renal corpuscles. The central or glomerular zone can be divided radially into the pars radiata, containing branching collecting tubules and straight thick portions of loops, and the pars labyrinthica, containing renal corpuscles and convoluted tubules. The innermost or subcortical zone is without corpuscles and thin portions of loops. In this area are found the distal portions of the proximal convoluted tubules, portions of the ascending limbs of Henle, and collecting tubules.

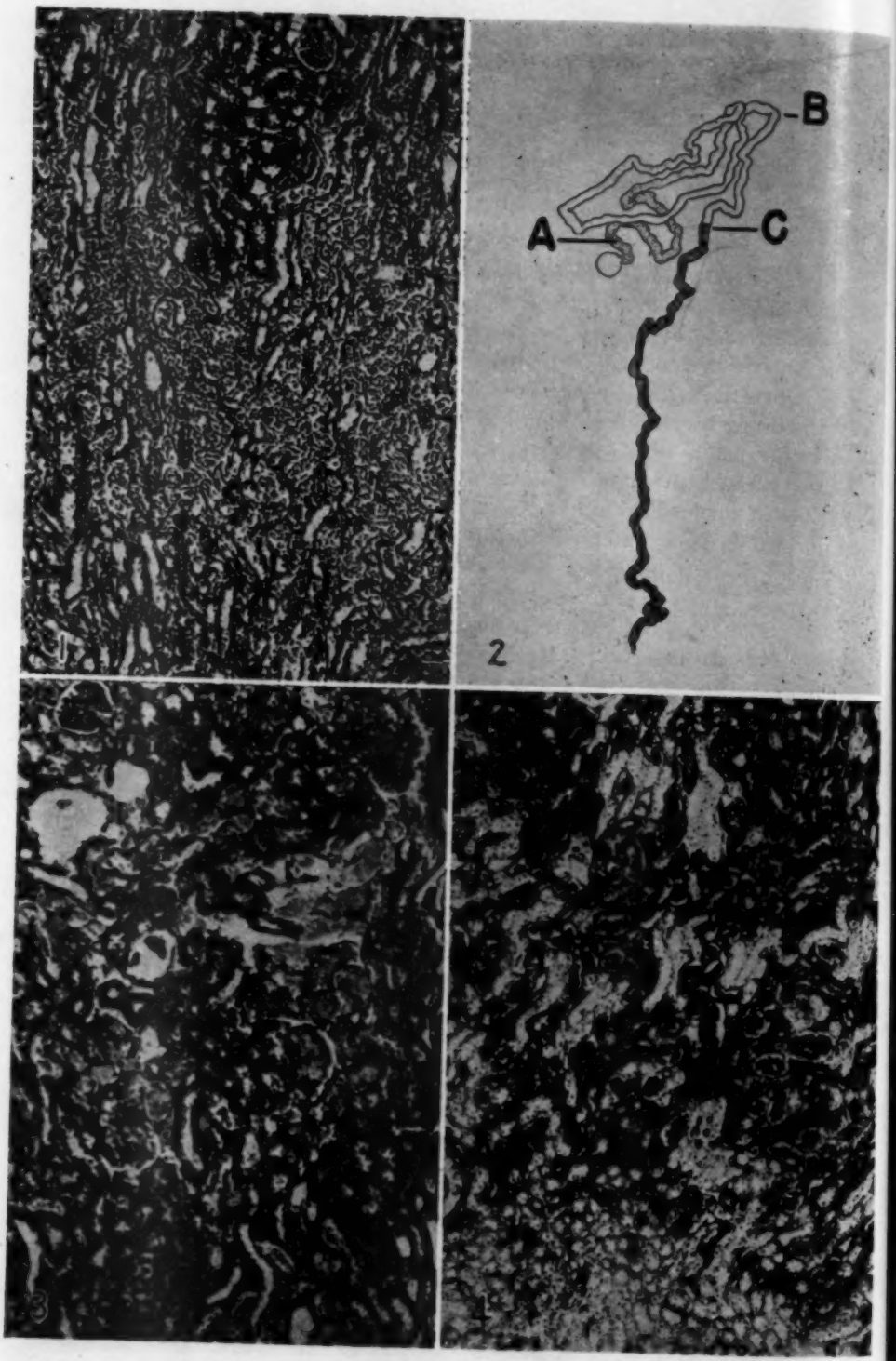
It is obvious, therefore, that the damage occurring after the administration of *dl*-serine may affect any of these three different structures.

10. Oliver, J., in Harvey Lectures, 1944-1945, Lancaster, Pa., Science Press Printing Company, 1945, vol. 40, p. 102.

11. Morehead, Fishman and Artom (footnotes 3 and 5).

12. Peter, K.: Untersuchungen über Bau und Entwicklung der Niere, Jena, Gustav Fischer, 1927, pp. 338 and 567.

13. Heidenhain, M.: Synthetische Morphologie der Niere des Menschen, Leiden, E. J. Brill, 1937.



Figures 1 to 4

(See legend on opposite page)

No definite decision could be made on the basis of sections stained with hematoxylin and eosin, although wherever damage was extensive the probability seemed strong that both ascending limbs of Henle's loop, as well as proximal convoluted tubules, were affected. In order to study this question further, a number of rats were given injections of lithium-carmine solution, as previously stated. Suzuki [14] introduced this method for the localization of tubular injury. Only the epithelium of the proximal convoluted tubules is able to concentrate the dye in particulate form. Dye particles are found in large amounts forming coarse granules in the uppermost segment of the proximal convoluted tubules. The cells of the median portion are less intensely stained, while the cells of the distal third contain only irregularly distributed dustlike dye particles. Using this method, Suzuki was able to show that uranium and mercury affected predominantly the distal portions of the proximal convoluted tubules, while chromium damaged the proximal portion. However, the application of this method was not quite satisfactory for the present problem. In normal control rats, five injections of lithium-carmine solution gave satisfactory staining in the proximal convoluted tubules located in the outer portion of the cortex. The distal portions of the proximal convoluted tubules in the subcortical zone showed only irregular and weak staining. In the animals previously treated with carmine and subsequently given *dl*-serine, a number of the necrotic tubules showed faint diffuse carmine staining. However, this was not observed regularly, and there was considerable variation in different animals. The only conclusion that could be drawn from these experiments was that distal portions of the

14. Suzuki, T.: Zur Morphologie der Nierensecretion unter physiologischen und pathologischen Bedingungen, Jena, Gustav Fischer, 1912.

EXPLANATION OF FIGURES 1 TO 4

Fig. 1.—Section of the kidney of a male rat fed the synthetic diet and killed twenty-four hours after administration of 100 mg. of *dl*-serine by stomach tube. There is extensive necrosis of tubular epithelium in the subcortical zone. Hematoxylin and eosin; × 75.

Fig. 2.—Camera lucida tracing of a proximal convolution isolated from the kidney of the rat furnishing the section shown in figure 1. *A*, scattered fine droplets between nuclei in the first third of the proximal convoluted tubule. The mitochondria are well preserved. *B*, beginning of the terminal segment of the proximal convolution. *C*, abrupt beginning of complete necrosis of the lower terminal segment. × 32.

Fig. 3.—Section of the kidney of a male rat fed the synthetic diet and killed thirty minutes after an intravenous injection of 100 mg. of *dl*-serine. Focal tubular necrosis may be seen. Hematoxylin and eosin; × 120.

Fig. 4.—A histotechnical preparation for the demonstration of alkaline phosphatase in the kidney of a rat killed twenty-four hours after an intraperitoneal injection of 50 mg. of *dl*-serine. The sites of enzymatic activity stain dark. The necrotic tubular epithelium in the subcortical zone contains phosphatase. Undamaged tubules in this location are devoid of cytoplasmic phosphatase. × 75.

proximal convoluted tubules were damaged by *dl*-serine. In view of the irregularity of the staining reaction no conclusion could be drawn as to the nature of necrotic tubules that did not contain carmine. An additional source of error may be a nonspecific absorption of excreted carmine by necrotic cells.

The isolation of single units permitted exact localization of the tubular damage. In view of the extremely severe destruction in the nephrons, the damaged portions consisted of nothing but basement membranes containing cellular debris. They therefore fell apart easily. However, a sufficient number of nephrons from three kidneys could be isolated, and the localization of the lesion was made quite clear. The lesion was localized exclusively in the terminal portion of the proximal convolution (fig. 2). No evidence of necrosis was observed in the ascending limb, the distal convolution or elsewhere. There were, however, casts in distal convolutions and more in collecting tubules, but the epithelium in these regions was undamaged. The upper part of the proximal convolution was well preserved. In most of the preparations scattered fine dark round bodies were seen, which were interpreted by Dr. Oliver as droplets of some material, possibly of protein nature. These droplets were more concentrated near the glomerulus and gradually faded out, to end, as one's observation descended the proximal tubule, at about the beginning of its second third.

Relation of Amount of dl-Serine to the Extent of the Renal Damage.—As previously stated, 100 mg. of *dl*-serine produced severe renal damage in all rats to which it was given. In addition, 30 rats fed a complete diet were given 50 mg. of *dl*-serine dissolved in 3 cc. of water intraperitoneally. They showed changes comparable to those seen with the larger dose of 100 mg., though in some instances the necrosis was less extensive. In order to establish the smallest dose that would produce demonstrable renal lesions, 48 rats were divided into two groups; 21 rats fed a normal diet and 27 fed the synthetic diet were given a single intraperitoneal dose of *dl*-serine, varying between 10 and 30 mg., in 3 cc. of water. The lesions observed were graded as ±, + and ++. Two pluses signified intense necrosis comparable to that seen with larger doses, although somewhat less extensive. Kidneys showing 1 plus lesions revealed areas of distinct necrosis. Here necrotic tubules in the inner portion of the cortex were frequently interspersed among undamaged tubules. Kidneys designated as having a plus-minus lesion showed only occasional frankly necrotic tubules. The damaged tubules showed more often fragmentation of the cytoplasm and pyknosis of nuclei.

As may be seen from table 1, a single dose of 10 mg. of *dl*-serine causes in most instances mild focal changes, while extensive necrosis was repeatedly observed following 20 to 30 mg. of *dl*-serine.

In 2 rats receiving intraperitoneally 500 mg. of *dl*-serine dissolved in 6 cc. of water the changes which occurred were essentially similar to those seen with 100 mg., although they appeared to be somewhat more extensive.

Relationship of Time to the Development of the Renal Lesion.—A rat killed incidentally ten hours after the administration of *dl*-serine

TABLE 1.—*Relation of the Amount of dl-Serine to the Extent of the Renal Changes*

Amount of dl-Serine, Mg.	Total Number of Rats	Number Fed the Complete Diet Who Showed				Total Number of Rats	Number Fed the Synthetic Diet Who Showed			
		No Lesions	± Lesions	+ Lesions	++ Lesions		No Lesions	± Lesions	+ Lesions	++ Lesions
10	1	1	0	0	0	6	1	5	0	0
15	4	2	1	1	0	9	0	1	6	2
20	4	1	2	0	1	8	1	1	0	6
25	6	0	1	1	4	2	0	1	1	0
30	6	0	3	3	0	4	0	0	1	3

revealed extensive necrosis indistinguishable from that seen after twenty-four hours. This observation prompted a study of the shortest time after which lesions of the kidneys would appear. For this purpose 45 rats were given the standard dose of 100 mg. of *dl*-serine; 20 animals received the drug intravenously, and 27, intraperitoneally. As

TABLE 2.—*Relationship of Time to the Development of the Renal Lesion*

Time Elapsing After Administration of dl-Serine Before Animals Were Killed, Minutes	Total Number of Rats	Number Showing Demonstrable Lesions
10	1 (1)*	0 (0)*
15	2 (2)	0 (0)
20	2 (2)	0 (0)
25	1 (1)	0 (0)
30	4 (4)	2 (2)
60	2 (1)	2 (1)
75	3 (2)	3 (2)
90	3 (1)	3 (1)
100	1 (1)	1 (1)
105	2 (1)	2 (1)
120	6 (1)	6 (1)
150	2	2
180	8 (1)	8 (1)
240	4 (1)	4 (1)
300	4 (1)	4 (1)
360	2	2

* The figures in parentheses are the numbers of rats given the dl-serine by intravenous injection.

may be seen in table 2, no definite lesions were observed earlier than thirty minutes after the intravenous injection. Among 4 rats 1 showed slight and 1 fairly marked evidence of renal injury thirty minutes after intravenous administration of *dl*-serine (fig. 3). All rats killed after seventy-five to three hundred and sixty minutes showed renal damage. In the early stages the damaged cells revealed often marked eosinophilia in their cytoplasm, while the nuclei appeared to be less intensely stained than those in uninvolved tubules. The cytoplasm

at the same time appeared shrunken, so that the height of the cell was less than that of cells lining uninvolved tubules. In many of the damaged proximal convoluted tubules, the brush border was separated from the eosinophilic cytoplasm and stood out clearly. In some of the tubules the cytoplasm showed considerable fragmentation, while the nuclei were still intact. Often the luminal portion of the cytoplasm seemed to break off, forming sometimes irregularly shaped eosinophilic clumps and at other times rounded, more basophilic structures. Lesions of this kind were mainly seen in the kidneys of rats killed up to three hours after injection, while in the following hours the damaged tubules showed more and more the picture of diffuse necrosis with complete loss of all structures of the cytoplasm and disappearance of the nuclei. However, distinct necrosis was seen in some of the tubules as early as thirty to sixty minutes after administration of *dl*-serine. The change was focal in the early stages and became more diffuse later on. Litter mates which received intraperitoneal and intravenous injections simultaneously and were killed after intervals varying from seventy-five to three hundred minutes revealed no significant differences in the extent and severity of the renal damage.

Influence of Weight.—Five adult male rats and 5 weanling rats were used. Three rats weighing between 170 and 190 Gm. received each 100 mg. of *dl*-serine subcutaneously. Two rats weighing 185 Gm. and 280 Gm., respectively, received 100 mg. of *dl*-serine for each 100 Gm. of body weight. In all 5 rats extensive necrosis was observed. No significant difference was noticed between those given the smaller and those given the larger dose. Of the 5 weanling male rats weighing 35 to 40 Gm., 2 were given 25 mg. and 3 were given 50 mg. of *dl*-serine. In 4 of these animals the extent of renal damage was similar to that seen in the standard 100 Gm. rat. The necrotic changes, although present, were slight in only 1 rat.

Influence of Sex.—Of 11 female rats weighing approximately 100 Gm., 5 were given 100, 2 were given 50 and 4 were given 30 Gm. of *dl*-serine intraperitoneally. Of the 5 rats receiving 100 mg., 4 showed renal damage fully comparable to that seen in male rats, while in 1 no lesions developed. In the 2 animals receiving 50 mg., extensive renal necrosis was observed. Of the 4 rats receiving 30 mg., 2 showed considerable, 1 slight and 1 no necrosis.

In addition, 6 adult female rats were given the *dl*-serine. Three weighing between 195 and 235 Gm. received 100 mg. by intraperitoneal injection. Of these, 2 showed considerable necrosis, while in 1 only slight degenerative changes and no renal necrosis was found. Three other rats weighing from 205 to 250 Gm. were given 100 mg. *dl*-serine per hundred grams of body weight. Of these, 1 showed marked and 1 moderate renal necrosis, and in 1 the kidneys remained unchanged.

Phosphatase Activity.—In the rat's kidney alkaline phosphatase activity is found predominantly in the cortical portion.[15] Cytoplasmic activity is seen mostly in the proximal convoluted tubules, while the tubular structures in the medulla show phosphatase in the nuclei only. Phosphatase activity is seen also in the glomeruli,[15d] in the adventitia of larger arteries and veins and in the capillaries of the medulla.

In 8 animals phosphatase activity was examined twenty-four hours after administration of *dl*-serine. The necrotic tubules still contained the enzyme. They were uniformly and deeply stained. A similar reaction was given by desquamated cell masses. Some of the casts also revealed enzymatic activity. Undamaged tubules devoid of cytoplasmic phosphatase in the subcortical zone were apparently more numerous than in sections of normal controls (fig. 4). No changes in phosphatase activity were noticed in any of the proximal convoluted tubules situated in the more peripheral portions of the renal cortex.

COMMENT

The results reported here fully confirm the findings of Morehead and co-workers [16] concerning the nephrotoxic action of *dl*-serine. When a dose of 100 mg. was given to male rats weighing 100 Gm. either by stomach tube or by intraperitoneal injection, all the animals showed typical renal necrosis at the corticomedullary junction within twenty-four hours. The damage was somewhat more severe in animals fed for seven days on a synthetic diet deficient in the B vitamins and poor in protein. Changes of similar degree and severity were seen in most rats given half the dose with only few exceptions, in which necrosis, although present, was less extensive. Five hundred milligrams *dl*-serine given in a single dose did not essentially influence the extent of the renal damage.

An attempt was made to establish the minimum amount of the amino acid capable of exerting a nephrotoxic action. As little as 10 mg. of *dl*-serine given to male rats weighing 100 Gm. and fed the synthetic diet led to focal degenerative changes, while 20 to 30 mg. produced extensive necrosis in a number of animals. Since only the unnatural *d*-isomer in the racemic mixture exerts apparently a nephrotoxic action,[6] the minimal kidney-damaging dose may be as low as 5 mg. In this connection the observations of Fishman and Artom [17]

15. (*a*) Gomori, G.: J. Cell. & Comp. Physiol. **17**:71, 1941. (*b*) Bourne, G.: Quart. J. Exper. Physiol. **32**:1, 1943. (*c*) Menten, M. L.; Junge, J., and Green, M. H.: Proc. Soc. Exper. Biol. & Med. **57**:82, 1944. (*d*) Wachstein, M.: Arch. Path. **38**:297, 1944; (*e*) J. Exper. Med. **84**:25, 1946.

16. Morehead, Fishman and Artom (footnotes 3 and 5).

17. Fishman, W. H., and Artom, C.: Proc. Soc. Exper. Biol. & Med. **60**:288, 1945.

are of interest, who found that 6 to 13 per cent of a 100 mg. dose of *dl*-serine is excreted unchanged in the urine.

The rapidity with which the anatomically demonstrable lesion may occur after administration of *dl*-serine merits some discussion. Evidence of renal damage was visible after only thirty minutes in a few instances, and degenerative changes were regularly noticed within sixty to seventy-five minutes. These degenerative changes increased in severity in the course of the next hours, and after four to five hours frank necrosis was quite extensive in most of the examined kidneys.

Only few reports could be found mentioning the earliest microscopic evidence following the administration of nephrotoxic substances. Kosugi [18] found evidence of tubular damage not earlier than six hours after the administration of mercury bichloride in rats. Ginzler,[19] however, stated that degenerative lesions could be seen in rabbits four hours after intravenous injection of mercury bichloride. After chromium had been administered, no microscopic lesions were seen within ten hours.[20] Hydropic changes of the epithelium of the convoluted tubules in rabbits occurred two and a half to five and a half hours after the injection of tartaric acid.[21] Within from one to two hours [22] after sodium oxalate had been administered intravenously to rabbits, necrosis was found in some of the convoluted tubules and even still earlier [23] with larger doses. Mitochondrial changes were seen in rabbits twenty minutes after the intravenous injection of uranium nitrate, while the first microscopic evidence of renal damage was noticed in sections stained with hemalum and eosin within two hours.[23]

More recently Gilman and co-workers [24] found sodium tetrathionate to be extremely nephrotoxic. Advanced renal necrosis was observed in dogs within thirty minutes after intravenous injection of this substance.

The nephrotoxic action of *dl*-serine is not restricted to rats weighing 100 Gm. but can be produced in weanling as well as in adult animals. Fishman and Artom [1] noticed a considerable resistance of female rats against the toxic effects of prolonged administration of *dl*-serine. However, renal lesions occur also in female rats, although not with the same regularity as in males. The fact that sex differences exist in

18. Kosugi, T.: Beitr. z. path. Anat. u. z. allg. Path. **77**:1, 1927.

19. Ginzler, A. M.: Personal communication to the author.

20. Eichler, O.: Handbuch der experimentellen Pharmakologie, Berlin, Julius Springer, 1924, vol. 3, pt. 3, p. 1526.

21. Potter, A. C., and Bell, E. T.: Am. J. M. Sc. **149**:236, 1915.

22. Dunn, J. S.; Haworth, A., and Jones, N. A.: J. Path. & Bact. **27**:299, 1924.

23. Gough, J.: J. Path. & Bact. **34**:423, 1931.

24. Gilman, A.; Philips, F. S.; Koelle, E. S.; Allen, R. P., and John, E. St.: Am. J. Physiol. **147**:115, 1946.

the kidney's reactivity to nephrotoxic influences has been previously established. The typical hemorrhagic necrosis which occurs in young rats fed choline-deficient diets is more common in males than in females.[25] After oral administration of chloroform, Eschenbrenner[26] found renal necrosis in male but not in female mice. Male rats showed a higher incidence of necrotizing nephrosis when exposed to repeated inhalation of carbon tetrachloride vapors than female animals.[27]

The peculiar location of the necrotizing lesion of the kidney suggested that a detailed analysis of the involved tubules be made. The general localization of the renal damage in the subcortical zone made obvious that the distal portion of the proximal convoluted tubules, the upper portion of the ascending loops of Henle and portions of the collecting tubules could be involved. Attempts to utilize the vital staining technic of Suzuki did not lead to unequivocal results. The most satisfactory technic for the localization of functional and pathologic processes in certain parts of the renal tubules, consists in the isolation of single renal units, as pointed out by Oliver.[10] With the help of this technic, Edwards[28] was able to demonstrate that mercury bichloride damages predominantly the second and third quarters of the proximal convoluted tubules, while the most distal portions were usually spared.

In isolated and appropriately stained nephrons the *dl*-serine damage was found to be localized in the distal portions of the proximal convoluted tubules, whereas all other structures were spared. The results of this technic is most revealing in view of the extensive involvement of the subcortical zone, which would lead one to believe that not only the proximal convoluted tubules but also the ascending limbs of Henle are involved in the morbid process. Morehead and co-workers[3] stated that necrosis was most marked in the innermost portion of the cortex but sometimes extended toward the periphery to involve the descending portions of the proximal convoluted tubules, obviously assuming that Henle's loops were predominantly injured. The findings reported here are in line with the well known susceptibility of the proximal convoluted tubules to various renal poisons.

In view of the fact that only the terminal portion of the proximal convoluted tubule is damaged, *dl*-serine should prove a valuable tool for producing a strictly localized renal lesion. In contrast to mercury and uranium, it does not involve other segments of the nephron regardless of the amount given. In addition, the lesions can invariably be produced with adequate doses in male animals.

25. Griffith, W. H.: J. Nutrition **19**:437, 1940.
26. Eschenbrenner, A. B.: J. Nat. Cancer Inst. **5**:251, 1945.
27. György, P.; Seifter, J.; Tomarelli, R. M., and Goldblatt, H.: J. Exper. Med. **83**:449, 1946.
28. Edwards, J. G.: Am. J. Path. **18**:1011, 1942.

In the kidney of the rat cytoplasmic phosphatase activity is marked in the cortex and in the subcortical zone. In the latter, most of the tubules show enzymatic activity, while only occasionally some are devoid of it. It still is an unsettled question whether the ascending loops of Henle participate in this reaction, as stated by Menten and co-workers.[15c] The experience with *dl*-serine clearly demonstrated that in tissue sections most of the tubules located in the subcortical zone are proximal convolutions. Since no cytoplasmic phosphatase activity is seen in the ascending limbs, located in the medulla, it seems reasonable to assume that these structures in the rat kidney do not show enzymatic activity. This circumstantial evidence is strengthened by the fact that after administration of *dl*-serine only necrotic tubules in the subcortical zone revealed phosphatase, while the cytoplasm of undamaged structures remained unstained. It must, however, be conceded that the final solution of this question will have to wait until isolated renal units can be examined with the phosphatase stain.

Cells made necrotic by the action of *dl*-serine contained the enzyme in the diffuse form. Similarly the necrotic cells in the acute phase of poisoning with uranium,[29] mercury bichloride [29a,b,d] and potassium dichromate [29a,b] still revealed enzymatic activity.

SUMMARY

The findings of Fishman, Artom and Morehead concerning the nephrotoxic action of *dl*-serine in rats were fully confirmed. Not only male animals weighing 100 Gm. but also weanling and adult rats are susceptible. In female rats *dl*-serine exerts likewise a nephrotoxic action, although less regularly.

The minimal injurious dose for male rats weighing 100 Gm. and fed a synthetic diet poor in the B vitamins and deficient in protein was found to be 10 mg., while 20 to 30 mg. led in most animals to extensive necrosis. Distinct renal lesions were observed in some instances as early as thirty minutes after intravenous injection of *dl*-serine.

By vital staining with a lithium-carmine solution and by using the maceration and dissection method of Oliver, the damage of the kidney was localized in the terminal portions of the proximal convoluted tubules. After the introduction of *dl*-serine, phosphatase activity was still found twenty-four hours later in the cells of necrotic tubules. The restriction of the damage to the distal portion of the convoluted tubules and its regular occurrence suggest that *dl*-serine would be a valuable tool for the production of localized renal injury.

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29. (*a*) Hepler, O. E.; Simmonds, J. P., and Gurley, H.: Proc. Soc. Exper. Biol. & Med. **44**:221, 1940. (*b*) Hepler, O. E.; Gurley, H., and Simmonds, J. P.: Arch. Path. **39**:133, 1945. (*c*) Breedis, C.; Flory, C. M., and Furth, J.: ibid. **36**:402, 1943. (*d*) Wachstein.[15e]

NEPHROTOXIC ACTION OF *DL*-SERINE IN THE RAT

II. The Protective Action of Various Amino Acids and Some Other Compounds

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IN THE preceding communication [1] the observations of Fishman, Artom and Morehead [2] concerning the injurious action of *dl*-serine on the rat kidney were fully confirmed. In amplification of these experiments it was found that not only male rats weighing 100 Gm. but weanling as well as adult animals are susceptible. Kidneys of female rats are likewise damaged, although less frequently. As little as 10 mg. may exert a nephrotoxic action in male rats weighing 100 Gm. Microscopically demonstrable renal damage may appear as early as thirty minutes after intravenous administration. The necrosis is limited to the terminal portion of the proximal convoluted tubules.

In agreement with Morehead, Fishman and Artom,[2b] renal changes were found to be more extensive on a synthetic diet poor in protein and deficient in B vitamins. These investigators found that addition of pure B vitamins to this diet reduced considerably the mortality and the severity of the clinical symptoms but did not influence the extent of the renal lesions.[3] Addition of choline hydrochloride, glycine and *l*-cystine to the diet had likewise no beneficial influence on the renal damage

The experiments reported here were started in view of the evidence brought forward recently that serine and methionine react in the animal body to form cystine.[4] It seemed of interest, therefore, to investigate whether methionine could influence the injurious action of *dl*-serine. Furthermore, it is known that renal lesions occur in weanling rats on

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1. Wachstein, M.: Arch. Path., this issue, p. 503.

2. (*a*) Fishman, W. H., and Artom, C.: J. Biol. Chem. **145**:345, 1942. (*b*) Morehead, R. W.; Fishman, W. H., and Artom, C.: Am. J. Path. **21**:803, 1945.

3. Fishman, W. H., and Artom, C.: Proc. Soc. Exper. Biol. & Med. **57**:241, 1944. Morehead, R. W.; Fishman, W. H., and Artom, C.: Am. J. Path. **22**:385, 1946.

4. (*a*) Stetten, D.: J. Biol. Chem. **144**:501, 1942. (*b*) Binkley, F., and du Vigneaud, V.: ibid. **144**:507, 1942. (*c*) Du Vigneaud, V.; Kilmer, G. W.; Rachele, J. R., and Cohn, M.: ibid. **155**:645, 1944.

various choline-free diets.[5] The renal changes are aggravated by simultaneous feeding of cystine and prevented by methionine.[6] Although the renal necrosis occurring in this condition is localized predominantly in the outer portion of the cortex and is of a hemorrhagic type, it seemed possible that the renal damage observed after *dl*-serine was due to the transformation of this substance into cystine.

In view of the beneficial action which *dl*-methionine was found to exert, a number of substances containing sulfhydryl groups, as well as other compounds, were tested for their protective influence.

MATERIAL AND METHODS

Litters containing 5 to 8 male albino rats of the Wistar strain, weighing about 100 Gm., were used for all experiments. In each litter 1 or 2 animals served as controls. In testing the protective action of *dl*-methionine, the animals were fed either "Rockland rat diet complete" (Arcady Farms, Milling Co., Chicago) or, for a period of seven days, a protein-deficient, vitamin B complex–free diet of a composition similar to that of the diet 1 used by Fishman and Artom.[2a] This diet consisted of vitamin test casein (General Biochemicals) 10 per cent, dextrin 39 per cent, sucrose 37 per cent, hydrogenated cottonseed oil ("crisco") 5 per cent, cod liver oil 5 per cent and salt mixture no. 2 U. S. P. XII 4 per cent. *dl*-Methionine was injected subcutaneously in doses of 100 mg. dissolved in 3 cc. of distilled water.

Animals protected with six doses of methionine received the amino acid morning, noon and evening on two consecutive days. On the second day the first dose of *dl*-methionine was given one hour before 100 mg. of *dl*-serine was administered by stomach tube. The next dose was given three to four hours later, and the last dose was given in the evening. Animals protected by five doses received the first injection at noon of the first day. Those protected with four doses of *dl*-methionine received it on the evening of the first day, while those receiving three doses of methionine were given it on the same day that *dl*-serine was administered. To those animals receiving two injections the *dl*-methionine was given one hour before and three to four hours after the test dose of *dl*-serine. Rats serving as controls received only 100 mg. of *dl*-serine by stomach tube and were treated with an equivalent volume of distilled water, injected subcutaneously.

Since no definite difference was seen in the methionine protection experiments between animals fed the complete and those fed the synthetic diet, the complete diet was used in the experiments with other compounds. *l* (+) Cysteine hydrochloride, sodium thioglycollate, glutathione, 2,3-dithiopropanol (BAL), *dl*-alpha-alanine, glycine, *dl*-threonine, *l* (+) glutamic acid, *l* (+) histidine monohydrochloride, *l* (+) arginine monohydrochloride, *dl*-valine, glycollic acid, butyric acid, pyruvic acid, sodium lactate, *d*-glucose, sodium chloride and sodium acetate were tested for their protective action.

In view of the fact elicited in the course of this work [1] that well within thirty minutes after the administration of *dl*-serine renal changes were microscopically demonstrable, the other tested compounds were given by three subcuta-

5. Griffith, W. H., and Wade, N. J.: J. Biol. Chem. **131**:567, 1939. György, P., and Goldblatt, H.: J. Exper. Med. **72**:1, 1940. Engel, R. W., and Salmon, W. D.: J. Nutrition **22**:109, 1941. Christensen, K.: Arch. Path. **34**:633, 1942. Wachstein, M.: ibid. **38**:297, 1944.

6. Griffith, W. H., and Wade, N. J.: J. Biol. Chem. **132**:627 and 639, 1940. Griffith, W. H.: J. Nutrition **21**:291, 1941.

neous injections, fifteen minutes before and fifteen and forty-five minutes after the intraperitoneal injection of a test dose of 50 mg. of *dl*-serine. The animals were likewise killed twenty-four hours later. *dl*-Serine was dissolved in 3 cc. of distilled water, while most of the tested substances were dissolved in 2 cc. of distilled water and the hydrogen ion concentration adjusted approximately to p_H 7.2. In most instances each injection contained 100 mg. of the tested substance. As in the previous experiments, at least 1 litter mate served as a control, receiving the same amount of *dl*-serine and injections of distilled water in equivalent amounts. Certain exceptions to this schedule were made, which will be referred to later.

RESULTS

Effect of dl-Serine.—The renal changes occurring in rats fed the complete and those fed the synthetic diet after the administration of *dl*-serine were described in the preceding paper.[1] They occurred regularly after the administration of 50 to 100 mg. of *dl*-serine. On gross

TABLE 1.—*Summary of the Results of Experiments to Determine the Extent to Which Various Doses of dl-Methionine Would Protect Rats Fed a Complete Diet Against Renal Injury Caused by Intragastric Administration of 100 Mg. of dl-Serine.*

Number of Subcutaneous Injections of dl-Methionine (100 Mg. in 3 Cc. of Water)	Total Number of Rats	Number of Rats Presenting		
		Maximal Lesions	Moderate Lesions	Slight or No Lesions
	18	18		
6	3	..	..	3
5	2	1	..	1
4	3	..	..	3
3	6*	..	2	4*
2	3	..	1	2
1	3	..	2	1

* Three rats received the three injections of dl-methionine within one hour.

examination a small, irregularly shaped strip of grayish discoloration which was seen at the innermost portion of the cortex indicated the necrotic area. Microscopically, the changes found were similar to those described by Morehead, Fishman and Artom.[2b] Acute renal necrosis occurred at the junction of the cortex and the medulla twenty-four hours after the administration of *dl*-serine. The epithelium of many tubules in this area was almost completely necrotic (figs. 1 and 3).

The changes observed varied somewhat in severity in different fields. Casts composed of eosinophilic granular material were frequent, located at times in the convoluted, but mostly in the collecting tubules. Not all the tubules at the corticomedullary junction were necrotic. Even where the damage was most extensive, occasional tubules were spared. In animals kept on the synthetic diet the renal damage induced by *dl*-serine was more extensive. In some fields the necrosis extended into the more outlying portions of the cortex. The glomeruli were not affected.

Effect of dl-Methionine on the Nephrotoxic Action of dl-Serine.—The influence of *dl*-methionine on the renal damage caused by *dl*-serine was examined in 42 rats. The results may be seen in tables 1 and 2.

"No lesions" and "slight lesions" signify complete absence of any damage (fig. 2) and the occasional degenerative changes noticed in some of the tubules in the inner portion of the cortex, respectively. In kidneys designated as showing moderate renal lesions, focal necrosis of cortical tubules was present. This necrosis was much less conspicuous than that seen in litter mates not protected by *dl*-methionine. In only 2 of all the animals was no protection found. The protective effect of *dl*-methionine was apparent in the animals on the protein-deficient diet as well as in those on the normal diet.

Effect of Some Other Amino Acids and Various Additional Compounds on the Nephrotoxic Action of dl-Serine.—The results of experiments on the protective action of some other amino acids and various additional substances against the nephrotoxic action of *dl*-serine are listed in table 3.

TABLE 2.—*Summary of Results of Experiments to Determine the Extent to Which Various Doses of dl-Methionine Would Protect Rats Which for Seven Days Had Been Restricted to a Protein-Deficient, B Vitamin–Free Diet Against Renal Injury Caused by Intragastric Administration of 100 Mg. of dl-Serine*

Number of Subcutaneous Injections of dl-Methionine (100 Mg. in 3 Cc. of Water)	Total Number of Rats	Number of Rats Presenting		
		Maximal Lesions	Moderate Lesions	Slight or No Lesions
	13	13		
6	4	..	..	4
5	3	..	..	3
4	3	..	..	3
3	4	..	..	4
2	3	..	..	3
1	5	1	2	2

1. *l* (+) Cysteine hydrocholoride. Of 10 animals given 100 mg. of *l* (+) cysteine hydrochloride three times, only 4 survived for a period of twenty-four hours. All of them showed the same amount of renal necrosis as the controls. Of 11 rats given 50 mg. of *l* (+) cysteine hydrochloride three times, 8 survived; 6 showed no protection; in 2 rats renal necrosis, although present, was less severe.

In order to test the possible damaging influence of cystine and cysteine, 8 normal rats restricted for seven days to the protein-deficient diet were given 45 mg. of *l* (—) cystine, and a second group of 8 normal rats received 90 mg. of *l* (—) cystine suspended in 3 cc. of distilled water daily by stomach tube for a period of seven days. These animals were killed on consecutive days. With exception of congestion of blood vessels in some of the kidneys, no lesions were found comparable to those occurring after administration of *dl*-serine.

Two normal animals fed the protein-deficient diet received two, and 3 normal animals received three administrations of 130 mg. of *l* (+)

cysteine hydrochloride in 3 cc. of water by stomach tube on consecutive days. In addition, 4 rats were given 150 mg. by subcutaneous injection. Necrotic changes were not found in the kidneys of any of these animals.

2. Sodium thioglycollate. Of 27 rats given 40 to 90 mg. of sodium thioglycollate by two subcutaneous injections (divided doses) administered within one hour, 15 survived twenty-four hours. The first dose, varying between 20 and 50 mg., was followed fifteen to thirty minutes

TABLE 3.—*Summary of Results of Experiments to Determine Whether Various Other Amino Acids and Some Other Compounds Would Protect Rats Fed a Complete Diet Against Renal Injury Caused by Intraperitoneal Injection of 50 Mg. of dl-Serine*

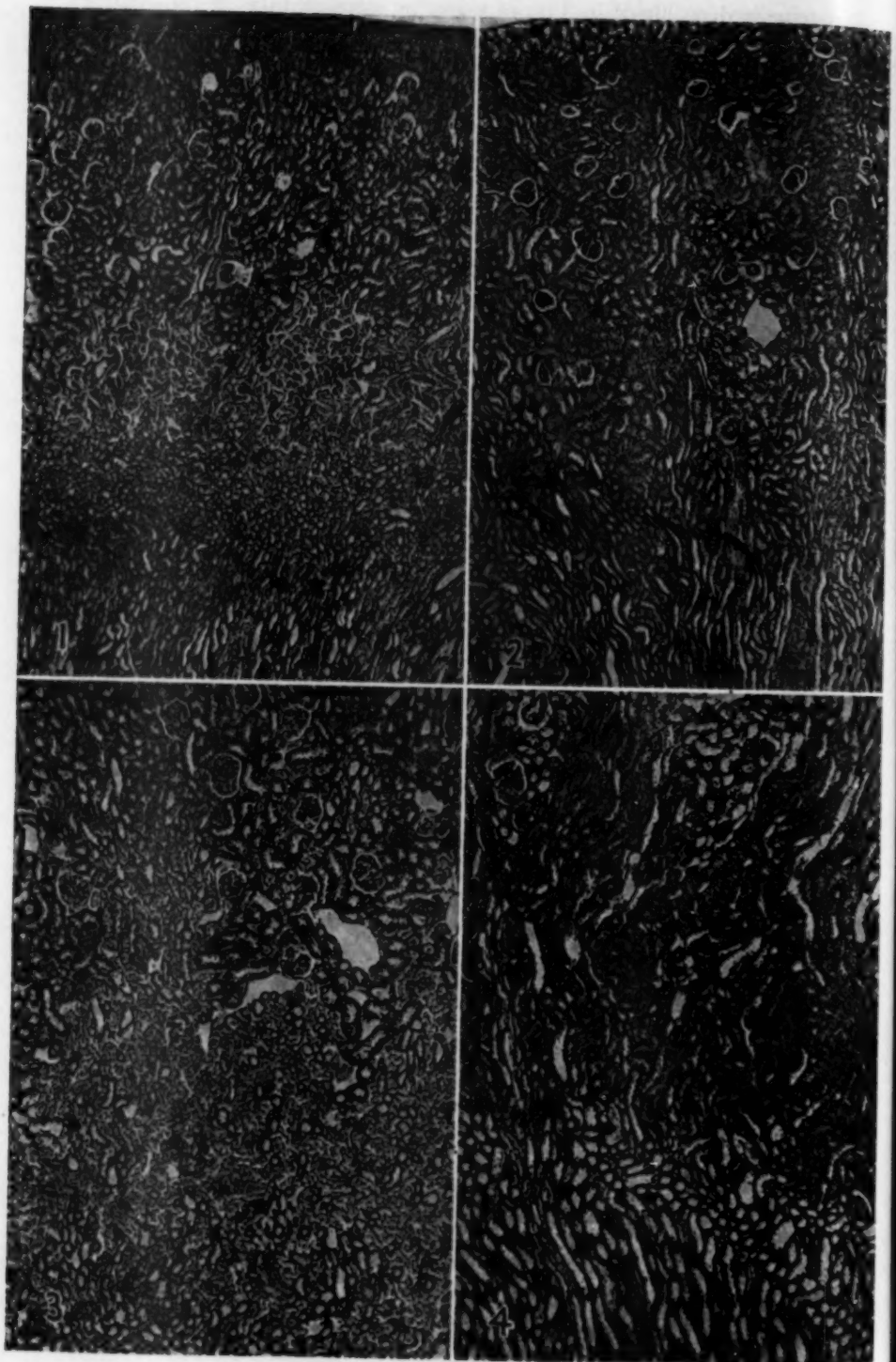
Substance Tested	Amount, Mg.	Mode of Injection	Total Number of Rats	Number of Rats Showing		
				Maximal Lesions	Moderate Lesions	Slight or No Lesions
			30	30		
l (+) Cysteine hydrochloride	300	Subcutaneous	4	4		
l (+) Cysteine hydrochloride	150	Subcutaneous	8	6	2	
Sodium thioglycollate	40	Subcutaneous	2	2		
Sodium thioglycollate	45	Subcutaneous	4	4		
Sodium thioglycollate	50	Subcutaneous	1	1		
Sodium thioglycollate	60	Subcutaneous	1	1		
Sodium thioglycollate	80	Subcutaneous	3	..	1	2
Sodium thioglycollate	90	Subcutaneous	4	3	1	
Glutathione	300	Subcutaneous	12	1	..	11
2,3-Dithiopropanol (Bal)	20	Intramuscular	1	1		
2,3-Dithiopropanol (Bal)	10	Intramuscular	2	2		
2,3-Dithiopropanol (Bal)	3	Intramuscular	3	3		
dl-Alpha-alanine	300	Subcutaneous	10	1	..	10
Glycine	300	Subcutaneous	10	1	..	9
dl-Threonine	300	Subcutaneous	9	..	..	9
l (+) Glutamic acid	300	Subcutaneous	3	1	1	1
l (+) Glutamic acid	150	Subcutaneous	5	5		
l (+) Histidine monohydrochloride	360	Subcutaneous	8	1	4	3
l (+) Arginine monohydrochloride	360	Subcutaneous	9	3	4	2
dl-Valine	300	Subcutaneous	8	2	5	1
Glycollic acid	180	Subcutaneous	12	..	1	11
Butyric acid	300	Subcutaneous	7	..	3	4
Pyruvic acid	300	Subcutaneous	7	..	2	5
Sodium lactate	300	Subcutaneous	7	1	6	
d-Glucose	300	Subcutaneous	7	7		
Sodium chloride	300	Subcutaneous	3	3		
Sodium acetate	300	Subcutaneous	5	5		

later by the test dose of *dl*-serine. The second dose of sodium thioglycollate in the amount of 20 to 40 mg. was given fifteen to fifty minutes later.

As may be seen from table 3, little protection was afforded by sodium thioglycollate. Only large doses, which were not tolerated by other rats, influenced the renal damage in some instances.

3. Glutathione. Of 12 animals given 100 mg. of glutathione three times, complete protection was afforded 11. Only in 1 animal was no beneficial effect seen.

4. 2, 3-Dithiopropanol (BAL). BAL in peanut oil containing 20 per cent benzyl benzoate (Hynson, Westcott & Dunning, Inc., Baltimore)



Figures 1 to 4.

(See legend on opposite page)

was used. Three animals were given one intramuscular injection of 0.1 cc. and 3 one intramuscular injection of 0.2 cc. containing 10 and 20 mg. of BAL, respectively. Thirty minutes later the test dose of *dl*-serine was administered. Two rats treated with the smaller dose, and 1 with the larger, survived for twenty-four hours. In addition, 3 other rats were given three injections of 1 mg. of BAL, the first dose being administered thirty minutes before the *dl*-serine, the second and the third dose fifteen and thirty minutes after. In none of these animals was any influence on the renal damage caused by *dl*-serine noticed.

5. *dl*-Alpha-alanine. Ten animals were given 100 mg. of *dl*-alpha-alanine three times. The kidneys of all of them were completely protected.

6. Glycine. Ten rats were given 100 mg. of glycine in three equal doses. In 9 of them complete protection occurred, while in 1 animal the necrotic lesions of the kidneys were not influenced.

In order that the influence of glycine itself on the kidneys might be examined, 4 normal rats were given 300 mg. of glycine dissolved in 3 cc. of distilled water. No appreciable renal lesions were found.

7. *dl*-Threonine. Nine animals were given 100 mg. of *dl*-threonine three times. All of them showed complete protection (fig. 4).

In view of the near chemical relationship of threonine and serine, 2 normal rats were given 100 and 3 were given 500 mg. of the substance intraperitoneally. No renal changes were seen.

8. *l* (+) Glutamic acid. Of 7 animals given 100 mg. of *l* (+) glutamic acid three times, only 3 survived. Six additional rats were given 50 mg. three times, of which 5 survived twenty-four hours. No influence was seen from the smaller dose, while the larger afforded some protection.

EXPLANATION OF FIGURES 1 TO 4

Fig. 1.—Section of the kidney of a male rat fed the synthetic diet and killed twenty-four hours after the administration of 100 mg. of *dl*-serine by stomach tube. There is extensive necrosis of tubular epithelium in the subcortical zone. Hematoxylin and eosin; × 37.5.

Fig. 2.—Section of the kidney of a litter mate of the rat furnishing the section shown in figure 1. This litter mate was protected by three subcutaneous injections of 100 mg. of *dl*-methionine and given the same amount of *dl*-serine. Necrotizing nephrosis is completely absent. Hematoxylin and eosin; × 37.5.

Fig. 3.—Section of the kidney of a male rat fed the complete diet and killed twenty-four hours after an intraperitoneal injection of 50 mg. of *dl*-serine. There is considerable necrosis of the tubular epithelium in the subcortical zone. Hematoxylin and eosin; × 55.

Fig. 4.—Section of the kidney of a litter mate of the rat furnishing the section shown in figure 3. This litter mate was protected by three subcutaneous injections of 100 mg. of *dl*-threonine. There is complete absence of renal necrosis. Hematoxylin and eosin; × 55.

9. *l* (+) Histidine monohydrochloride. Of 8 animals protected with 120 mg. of *l* (+) histidine monohydrochloride three times, 3 showed no renal lesions, while in 4 necrosis, although present, was less marked than in the unprotected controls. In 1 animal no protection was seen.

10. *l* (+) Arginine monohydrochloride. Of 9 animals given 120 mg. of *l* (+) arginine monohydrochloride three times, 3 showed severe renal lesions, while moderate protection was found in 4 and complete in 2.

11. *dl*-Valine. Eight rats received 100 mg. of *dl*-valine three times. No protection was seen in 2, while protection was complete in 1 and moderate in 5.

12. Glycollic acid. Of 12 rats given 60 mg. of glycollic acid three times, 11 showed complete protection, while in 1 the protection was less complete.

13. Butyric acid. The hydrogen ion concentration of the butyric acid could be adjusted to only about p_H 4.2. Of 7 rats given 100 mg. three times, no renal lesions were found in 4 and moderate lesions were found in 3.

14. Pyruvic acid. Of 7 rats given three equal doses of 100 mg. of pyruvic acid, 5 showed complete absence of the typical lesions of the kidneys, and 2 showed moderate lesions. However, there was a varying amount of hydropic degeneration in the proximal convoluted tubules located in the outer portion of the cortex. In 3 normal rats given 300 mg. of pyruvic acid dissolved in 6 cc. of distilled water similarly, some hydropic degeneration in the proximal convoluted tubules was seen. Necrotic changes were completely absent.

15. Sodium lactate. Of 7 animals given three injections of 100 mg. of sodium lactate, no protection was seen in 1, while moderate protection was found in 6.

16. *d*-Glucose. Seven animals received three doses of 100 mg. of *d*-glucose. No influence on the extent and severity of the renal lesions was seen.

17. Sodium chloride. In 3 rats given 100 mg. of sodium chloride in three doses, the extent of the renal lesions was not influenced.

18. Sodium acetate. Five animals given 100 mg. of anhydrous sodium acetate in three equal doses revealed renal changes that were not different from those of the controls.

COMMENT

A considerable protection of methionine as well as of other substances against the nephrotoxic action of *dl*-serine could be demonstrated in these experiments.

A protective action of methionine against the chloroform injury of the liver in protein-depleted dogs [7] and normal dogs [8] has been previously established. Methionine was shown to exert a beneficial effect on the hepatic injury in protein-depleted dogs given oxophenarsine hydrochloride ("mapharsen").[9] It also protected mice fed a complete [10] and rats fed a protein-deficient diet [11] against dichloroethane poisoning. Methionine failed to exert a protective action in rats and dogs on a complete diet poisoned with carbon tetrachloride.[12] György and co-workers [13] found that methionine and methionine-containing proteins protected against renal injury more consistently than against hepatic damage in rats exposed to the inhalation of carbon tetrachloride. *dl*-Methionine prevents to a considerable extent the severe degenerative changes induced in the liver and the kidneys of young rats by pyridine.[14]

Of other substances containing a sulfhydryl group tested, little effect from sodium thioglycollate and from cysteine and none from BAL was seen. BAL given in appropriate amounts prevents completely the renal injury occurring in mercury bichloride poisoning [15] and following the administration of sodium tetrathionate.[16] It can therefore be reasonably assumed that the serine damage is not due to interference with SH groups and that the protective action of methionine and glutathione, the other effective sulfhydryl-bearing substances tested, is not due to their SH group.

Glutathione is a tripeptide of glutamic acid, cysteine and glycine. Of these three substances, cysteine, as already stated, had little protective action. *l* (+) Glutamic acid had only moderate protective influence.

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13. György, P.; Seifter, J.; Tomarelli, R. M., and Goldblatt, H.: J. Exper. Med. **83**:449, 1946.

14. Baxter, H.: J. Clin. Investigation **25**:908, 1946.

15. Ginzler, A. M.: Federation Proc. **5**:221, 1946. Gilman, A.; Allen, R. P.; Philips, F. S., and John, E. St.: J. Clin. Investigation **25**:549, 1946.

16. Gilman, A.; Philips, F. S.; Koelle, E. S.; Allen, R. P., and John, E. St.: Am. J. Physiol. **147**:115, 1946.

Glycine, however, was effective. This is apparently in contrast to the observation of Fishman and Artom,[8] who did not observe a beneficial influence of the glycine added to the synthetic diet. *dl*-Serine, however, in a similar manner will damage the kidney only if given by stomach tube or intraperitoneally but not if added to the diet.[17] In addition, the amount of glycine injected was considerably larger.

Of other substances tested, *dl*-alanine, *dl*-threonine and glycollic, butyric and pyruvic acids, likewise were effective in preventing the *dl*-serine-produced renal injury. Pyruvic acid suppressed the renal necrosis induced by *dl*-serine, although it caused a moderate degree of hydropic degeneration in segments of the convoluted tubules located in the outer portions of the renal cortex. *l* (+) Histidine monohydrochloride and lactic acid afforded appreciable, while *l* (+) arginine monohydrochloride and *dl*-valine afforded moderate, protection. Glucose, sodium acetate and sodium chloride were without influence.

The number of substances tested had to be limited, of necessity. However, it is likely that other compounds can be found capable of influencing the renal injury induced by *dl*-serine.

Artom and Fishman postulated that the injurious action of *dl*-serine might be due to a mass action of its unmetabolized molecule or to the accumulation of products of its metabolism.[17] They later found the unnatural *d*-isomer to be injurious, while *l*-serine was not toxic.[18]

In view of the accumulation of bisulfite-binding substances in the urine of animals poisoned with *dl*-serine and the evidence brought forward that serine might be converted into pyruvic acid,[19] Fishman and Artom [20] considered the accumulation of pyruvic acid in the kidney as a possible cause of the renal injury. However, as previously stated, pyruvic acid has considerable protective action and, although producing some hydropic changes, it does not lead to renal necrosis.

As mentioned before, cystine can be formed from serine in the body of the rat.[21] Cystine was found to exert a nephrotoxic action if given intravenously to dogs in large amounts.[22] It has an aggravating influence on the renal damage caused by choline deficiency.[6] Neither in rats fed the normal diet nor in those fed the synthetic diet, however, did

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18. Artom, C.; Fishman, W. H., and Morehead, R. P.: Proc. Soc. Exper. Biol. & Med. **60**:284, 1945.

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20. Fishman, W. H., and Artom, C.: Proc. Soc. Exper. Biol. & Med. **60**:288, 1945.

21. Stetten.[4a] Binkley and du Vigneaud.[4b] Du Vigneaud and others.[4c]

22. Newburgh, L. H., and Marsh, P. L.: Arch. Int. Med. **36**:682, 1925.

large sublethal amounts of cystine or cysteine produce significant lesions of the kidneys.

A considerable fraction of serine is transformed into glycine in the rat and the guinea pig.[23] Glycine itself is completely innocuous and exerts strong protective action. It is of particular interest that only the naturally occurring *l*-serine is converted into glycine.[23] Similarly only the *l*-isomer can be converted into cystine by liver tissue.[4b] In view of these facts the first hypothesis of Fishman and Artom appears more likely, namely, that the damaging action of *dl*-serine is due to the presence of the unmetabolized *d*-isomer.

At normal plasma concentrations the reabsorption of amino acids from the glomerular filtrate is practically complete, and only small amounts are excreted in the urine. Elevation of the plasma concentration above the normal by the administration of amino acids leads to an increase in the rate of tubular reabsorption. If the process of reabsorption fails to keep pace with the increased rate of amino acid filtration by the glomeruli, increased amounts are excreted in the urine.[24] Pitts [24b] showed that creatine is reabsorbed by a mechanism similar to that which is active in the absorption of various amino acids. Competition between creatine and amino nitrogen for a common link in the reabsorptive chain leads to the reduction in the amount of creatine absorbed at elevated plasma amino acid levels. Glycine, *dl*-alanine and *l* (+) glutamic acid suppress the reabsorption of creatine. In contrast, *l* (+) arginine does not influence the tubular reabsorption of creatine. On the other hand, glycine was found to depress the reabsorption of *l* (+) arginine.

In the light of these findings it seems possible that the protective action of the various amino acids and other compounds against the nephrotoxic action of *dl*-serine is due to the competitive action of these substances on the reabsorption of the injurious *d*-isomer. Since the damage caused by *dl*-serine occurs only in the terminal segments of the proximal convoluted tubules,[1] it can be assumed that reabsorption of this amino acid occurs predominantly at this location. In contrast, glucose is reabsorbed in the first portions of the proximal convoluted tubules.[25]

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24. (*a*) Doty, J. R.: Proc. Soc. Exper. Biol. & Med. **46**:129, 1941. (*b*) Pitts, R. F.: Am. J. Physiol. **140**:156, 1943; **140**:535, 1944. (*c*) Goettsch, E.; Lyttle, J. D.; Grim, W. D., and Dunbar, P.; ibid. **140**:688, 1944. (*d*) Eaton, A. G.; Ferguson, E. P., and Byer, F. T.: ibid. **145**:491, 1946. (*e*) Beyer, K. H.; Wright, L. D.; Russo, H. T.; Skeggs, H. R., and Patch, E. A.: ibid. **146**:330, 1946. (*f*) Silber, R. H.; Seeler, A. O., and Howe, E. E.: J. Biol. Chem. **164**:639, 1946.

25. Walker, A. M., and Oliver, J.: Am. J. Physiol. **134**:562, 1941.

SUMMARY

The severe necrotizing nephrosis induced by *dl*-serine can be favorably influenced by several amino acids and related compounds. *dl*-Methionine and glutathione exert a considerable protective influence. This is probably not due to SH groups, since cysteine and thioglycollic acid have only little and 2, 3-dithiopropanol (BAL) has no beneficial effect. *dl*-Alpha-alanine, glycine, *dl*-threonine and glycollic, butyric and pyruvic acids have a considerable protective influence. *l* (+) Histidine monohydrochloride and lactic acid afford appreciable, *l* (+) arginine monohydrochloride and *dl*-valine moderate, protection. Some influence was seen from *l* (+) glutamic acid, while glucose, sodium acetate and sodium chloride were without effect. Neither cysteine nor pyruvic acid nor glycine, substances which can be formed from serine in the body of the rat, cause necrotizing nephrosis.

It is assumed that the beneficial effect of the various amino acids and other substances against the nephrotoxic action of *dl*-serine is due to their competitive suppression of tubular reabsorption of the injurious *d*-isomer.

Case Reports

FIBROMYOSIS UTERI: ENDOMETRIAL TYPE

J. H. HILL, M.D., KANSAS CITY, MO.

A UTERINE growth which resembles adenomyoma but which does not contain glands occurs with considerable rarity. For that reason the study of another case is justified. Goodall [1] called this type of growth "stromatous endometriosis," but the name is somewhat of a misnomer because no glands are seen in this condition. It would seem that the term "fibromyosis uteri, endometrial type" more accurately describes the condition. Frank [2] suggested the term "fibromyosis" for a similar condition, but this term does not sufficiently indicate the endometrial character of the connective tissue growth.

The first case was described as one of adenomyosis with stroma but no glands by Casler [3] in 1920. Between 1937 and 1941 Goodall brought this condition into prominence by his reports of 8 cases. His monograph must be consulted for all his opinions regarding endometriosis. In 1942 Robertson and associates [4] studied 6 cases of what they described as "benign stromal endometriosis" and a seventh case of growth clearly cancerous in character. They expressed the belief that adenomyosis and stromal endometriosis are variants of the same process but that the lesions of stromal endometriosis, unlike those of adenomyosis, may continue to grow after the cessation of ovarian function. Three cases were reported by Miller and Tennant [5] in 1944. In 1945 de Carle [6] reported 3 cases. Quite recently Henderson [7] has presented a clinical and pathologic study of 7 cases; to this condition he has given the name "endolymphatic stromal myosis." I have personal knowledge of 2 unreported cases, and in the literature are references to 3 other unreported cases. This condition may be somewhat more common than the literature would indicate.

REPORT OF A CASE

E. E. W., a 46 year old white woman, was admitted to the service of Dr. John Ogilvie at the Trinity Lutheran Hospital on May 1, 1946, complaining of profuse menstrual bleeding and a lump in the lower part of the abdomen. The

From the departments of pathology of the University of Kansas School of Medicine and the Trinity Lutheran Hospital.

1. Goodall, J. B.: Tr. Am. A. Obst. **50**:192, 1938; J. Michigan M. Soc. **39**:480, 1940; J. Obst. & Gynaec. Brit. Emp. **47**:13, 1940; A Study of Endometriosis, Endosalpingiosis, Endocervicosis and Peritoneo-Ovarian Sclerosis, ed. 2, Philadelphia, J. B. Lippincott Company, 1944.

2. Frank, R. T.: Am. J. Cancer **16**:1326, 1932.

3. Casler, D. B.: Surg., Gynec. & Obst. **31**:150, 1920.

4. Robertson, T. D.; Hunter, W. C.; Larson, C. P., and Snyder, G. A. C.: Am. J. Clin. Path. **12**:1, 1942.

5. Miller, J. R., and Tennant, R.: Am. J. Obst. & Gynec. **47**:784, 1944.

6. deCarle, D. W.: West. J. Surg. **53**:48, 1945.

7. Henderson, D. N.: Am. J. Obst. & Gynec. **52**:1000, 1946.

menorrhagia had begun seven months before admission and had become progressively more severe. One month before admission she suffered abdominal pain described by her as like "a green apple bellyache." At that time she noticed a lump in the lower part of the abdomen. She has 3 children aged 23, 21 and 18 years. Significant physical findings consisted of a firm, movable, nontender mass "about the size of a grapefruit," the upper surface of which could be palpated half way between the symphysis pubis and the umbilicus. Laboratory examination revealed moderate secondary anemia. The clinical diagnosis was uterine myoma. A supracervical amputation of the uterus was performed, with convalescence uneventful. When seen eight months after operation the patient was well except for hot flushes and nervousness requiring estrogenic therapy.

Fig. 1.—Drawing of a horizontal cross section of the uterus illustrating the assymetric enlargement of the uterus caused by intramural masses of stromal cells. *A* indicates the uterine cavity lined by unchanged mucosa; *B*, the narrow rim of compressed myometrium; *C*, an irregular focus of necrosis; *S*, stromal cell masses. × 0.92.

The specimen consisted of a spherical uterine tumor mass approximately 12 cm. in diameter. Gross section revealed the uterine cavity to be small and deflected far to one side by a tumor-like growth of one wall. This enlargement consisted of interlacing muscle bundles enclosing islands of pale, soft cellular tissue. In the fixed material (fig. 1) the muscle bundles contracted and the rubbery cellular islands bulged above the cut surface. Toward the center of the thickened wall was an irregularly shaped, reddish brown mottled area of softening approximately 3 cm. in diameter. The endometrium averaged 4 mm. in

thickness and had a smooth surface. Attached to the uterine fundus was a pedunculated subserous fibromyoma 4 by 3 by 3 cm. in size. The uterine tubes were congested. The ovaries were not enlarged; both were firm and contained numerous scars, and one bore a mature corpus luteum.

Microscopic sections from the thickened uterine wall showed broad masses of cells infiltrating between the bundles of smooth muscle (fig. 2 *A*); in places the cell masses grew into and along dilated lymphatic channels (fig. 2 *C* and *D*). The cells tended to be uniform in size and shape with oval, moderately vesicular nuclei that occasionally showed mitotic figures; the cytoplasm was so scanty as

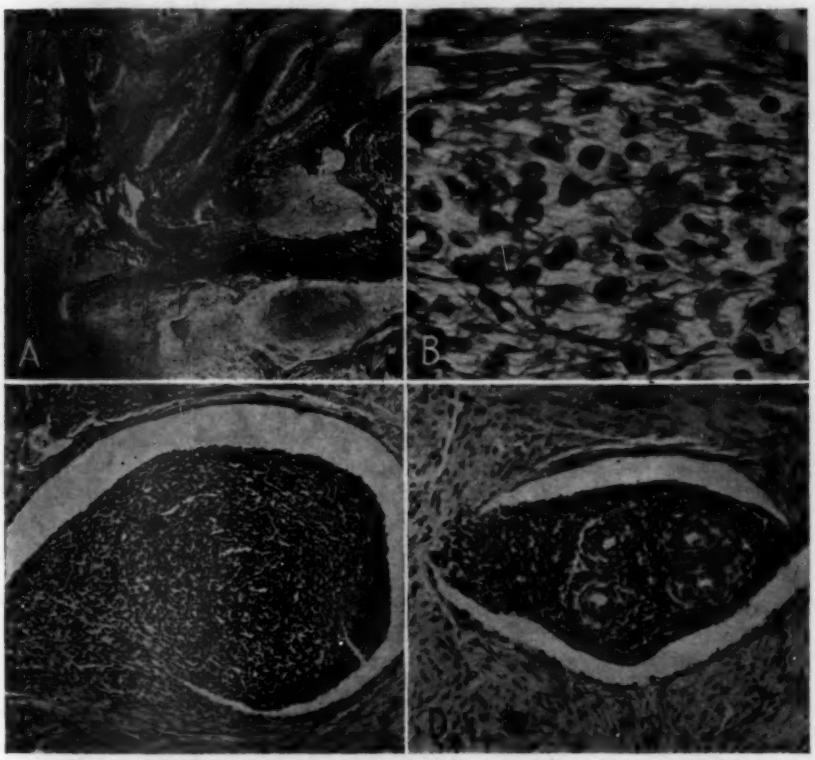


Fig. 2.—*A*, low power photomicrograph of a section of thickened uterine wall, illustrating the diffuse infiltrative type of stromal cell growth. Note muscle bundles (*m*) surrounded by stromal cell masses (*s*). Aniline blue-azocarmine; × 6.5.

B, photomicrograph showing diffuse network of reticulum fibers more or less intimately related to each stromal cell. Note the endothelial tube (*a*) and the uniformity of the nuclei of the stromal cells. Fine short strands of collagen can be demonstrated in the field with aniline blue and light green. Wilder's reticulum stain. × 591.

C, photomicrograph showing a mass of stromal cells in a dilated lymph vessel. Note that the mass is broadly attached to the vessel wall and that the intrinsic vascular tubes are apparently connected with the lumen of the lymph vessel. Hematoxylin-eosin; × 85.

D, photomicrograph showing four arteriole-like vessels contained in an endolymphatic mass of stromal cells. The surrounding myometrium is hyperplastic. Hematoxylin-eosin; × 110.5.

to be almost invisible. Reticulum stains revealed abundant reticulum formation with delicate argyrophilic fibrils intimately related to and surrounding each cell (fig. 2 *B*). Sections stained by the Masson technic revealed occasional inconspicuous collagen fibrils chiefly about the intrinsic vessels of the tumor. Elastic fibers were not demonstrated. The cells making up the invading masses were apparently derived from the endometrial stroma.

The vascular component of the cell masses was of considerable interest. Some vessels were obviously myometrial arteries that had been surrounded by the growing cells. Present throughout the specimen were small arteriole-like blood vessels having a concentrically arranged sheath of stromal cells. Reticulum stains showed a perivascular condensation of argyrophilic fibrils, some of which also stained for collagen; smooth muscle cells could not be demonstrated. These arteriole-like vessels sometimes occurred in small groups accompanying the growing cell masses into and along dilated lymph vessels (fig. 2 *C* and *D*); such vessels were formed by the stromal cells themselves. Other irregular, slightly dilated vascular spaces lined by flattened endothelium were present; those containing erythrocytes were considered to be venules, while the empty ones were lymph vessels.

Sections of the softened areas revealed foci of necrosis. Close to the foci of necrosis the stromal cells showed swelling of the cytoplasm and nuclear change, while other areas showed much interstitial edema and separation of the cells, resulting in a myxoma-like appearance. In some places the stromal cells were indistinct except for those around blood vessels, producing a perithelioma-like appearance.

Sections from the relatively intact, though thickened, uterine wall showed early secretory phase endometrium, while the underlying myometrium showed mild hyperplasia but no evidence of stromal cell growth. Section of one cornu showed slight adenomyosis of the usual type. Section of the broad ligaments revealed no evidence that tumor cells were extending beyond the limits of the uterus.

Some portions of the neoplastic tissue of the uterus showed rather thick bundles of moderately enlarged smooth muscle cells; the microscopic appearance together with the gross anatomy indicated myometrial hyperplasia.

The pathologic diagnosis was: fibromyosis uteri; endometrial type; focal cornual adenomyosis; subserous fibromyoma.

COMMENT

After studying this case and those previously reported, I do not believe that these cases should be called instances of endometriosis, because the stromal cell growth does not contain glands and in some cases at least the growth persists and causes death after ovarian function has been abolished. I have employed Frank's term "fibromyosis" to describe the growth observed in the present case, adding the phase "endometrial type" to indicate the specific endometrial stroma type of fibrogenic growth which characterizes this condition. While the stromal cells are actively reticulogenic and form only collagen about the intrinsic blood vessels, I think the "fibro–" is justified in view of the intimate connection known to exist between the argyrophilic reticulum and collagen; furthermore, McDonald and associates[8] concluded that endometrial stroma sarcoma was to be viewed as a variety of fibrosarcoma

8. McDonald, J. R.; Broders, A. C., and Counseller, V. S.: Surg., Gynec. & Obst. **70**:223, 1940.

since the growth sometimes forms collagen. The "myosis" is necessary to indicate the associated hyperplasia of smooth muscle which accompanies the stromal cell masses. Since the stromal cell of the endometrium is unique, I feel that the qualifying phrase "endometrial type" is required to indicate the specific cell type comprising the fibrogenic tissue.

As used by Goodall the term "endometriosis" implies a growth that may be composed of glands and stroma or of stromal cells alone. Ordinarily the term "endometriosis" implies the presence of both glands and stroma, their proportions varying within considerable limits; in the absence of either stroma or glands it is not usually believed that endometriosis is present. Furthermore, there is insufficient evidence to overthrow the view that the epithelium plays the dominant and formative role while the stroma plays a subordinate role. Novak[9] stated that the endometrial stroma was to be looked on as a satellite tissue appearing as the natural accompaniment of celomic epithelium which has reached a certain degree of differentiation. The differentiation existing between the stroma and the epithelium of endometrium, according to Gilbert,[10] is of a different order from that between the stroma and the epithelium of other organs; this together with the fact that they are both sensitive to endocrine secretions accounts, in his opinion, for some of the peculiarities seen in endometrial stroma growths. Proof that the normal form and function of the epithelium require contact or working together with the endometrial stroma has been offered by de Josselin de Jong.[11] He showed that when the glandular epithelium came in contact with fibrous connective tissue the epithelial cells became flattened so as to resemble endothelium. A study of decidua shows that the epithelium may become so flattened as to cause difficulty in distinguishing the glands from lymph channels or from venules.[12] These two observations indicate that the kind of supporting stroma may radically affect the appearance of the glandular epithelium, causing it to resemble endothelium. With this in mind I examined the sections of the present specimen in an effort to determine whether or not some of the endothelial-lined spaces could represent altered endometrial glands and thus allow the growth to be classified as a sort of atypical endometriosis. In this I was unsuccessful; that is, transition between glandular epithelium and endothelium could not be made out.

I believe these stromal cell growths arise from mesenchymal cell rests laid down during the development of the müllerian duct structures. Evidence that patches of mesenchymal cells do occur in various locations in the female pelvis and that they are sensitive to endocrine stimulation is afforded by a study of ectopic decidua islands.[13] I believe that these rests may occur in the myometrium and that an endocrine stimulus accounts for hyperplasia in the direction of the endometrial stromal cell and for the myometrial hyperplasia. The resultant

9. Novak, E.: Am. J. Obst. & Gynec. **12**:484, 1926.

10. Gilbert, J. B.: Clin. Proc. **2**:214, 1943.

11. de Josselin de Jong, R.: Schweiz. med. Wchnschr. **65**:197, 1935.

12. Novak, E.: Gynecological and Obstetrical Pathology, Philadelphia, W. B. Saunders Company, 1940, p. 123.

13. Novak,[12] p. 124.

growth is to be looked on as a hamartoma possessing a limited capacity for growth; it is probable that the hyperplasia may sometimes assume autonomous growth resulting in what amounts to a type of low grade fibrosarcoma.

In diagnosis the growth must be differentiated from endometrial sarcoma, leiomyosarcoma and anaplastic carcinoma of the endometrium. It may be excluded on the finding of polymorphism, numerous mitotic figures and destructive invasion of neighboring tissues.

The 3 cases reported by Frank under the title "'Fibromyosis': An Unclassified Plexiform Endolymphatic Proliferation of the Uterus" differ from the present case in that the tumor cells were largely confined within lymph channels and extended beyond the limits of the uterus. However, I think that both processes are basically similar. Those patients in whom all the growth is contained within the uterus are probably cured by operation, while those showing extension into the parametrium will probably suffer recurrence. It must be emphasized that the long latent period reported in some of the cases showing recurrence requires that ten to twenty year follow-up studies be made before statements of cures may be regarded as conclusive.

SUMMARY

A case of fibromyosis, endometrial type, is reported. It is believed that classifying growths of this type as variants of endometriosis is unwarranted. The term "fibromyosis," suggested by Frank, is more desirable when coupled with the qualifying phrase "endometrial type."

Obituaries

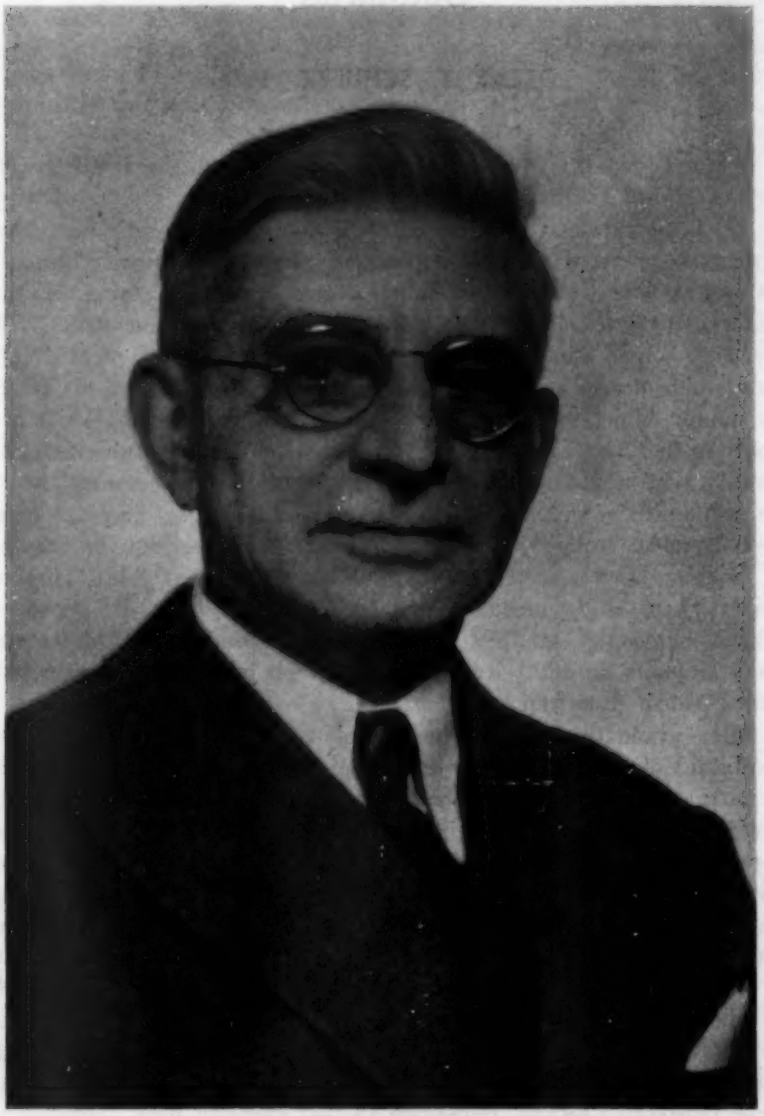
OSCAR T. SCHULTZ, M.D.

1877-1947

Oscar T. Schultz lived, until his college years, in the small farming town of Mount Vernon, Ind. He was the first of four children born to Dr. O. T. Schultz and Louise Pfeffer Schultz and was early introduced to the amosphere of a country general practice. Dr. Schultz Sr. was from a long line of doctors extending over several generations and thus it was natural for his son to take up the study of medicine. The precollege school education of Dr. Schultz Jr. was supplemented by a considerable degree of discipline and training supervised by his father, who taught him Greek and encouraged the use of German, which was spoken in that family and by O. T. from early childhood, and which later proved to be a great advantage. Dr. Schultz Sr. recognized in his son certain retiring and shy qualities and encouraged him to pursue the nonclinical studies in medicine rather than to engage in practice. Dr. Schultz Jr. was a "Hoosier" until he went to the Johns Hopkins University to study medicine, for he acquired his A. B. degree at Indiana University in 1897. Two years elapsed before he entered Johns Hopkins, in 1899—two years in which he served as a sergeant in the Spanish-American War.

After graduating from Johns Hopkins in 1903, he spent a few months at Columbia University and at the Brooklyn Institute. However, his career was firmly established in Cleveland, where he successively served as resident pathologist in the Charity Hospital and demonstrator in pathology and protozoology at Western Reserve University, then as instructor and lecturer, and finally as associate professor in pathology from 1910 to 1913 at the same university. Meanwhile he had married Irene Throop, with whom he had grown up in Mount Vernon, and with his wife had three children: Dr. Katherine, Louise and Oscar Jr. In 1913 he became professor of pathology and bacteriology at the University of Nebraska Medical School and remained there until 1916. In that year he became director of the Nelson Morris Institute of Michael Reese Hospital, Chicago, remaining in that capacity until 1929. This period was interrupted by a two year service in the Army as captain in the medical corps, a considerable portion of which time was spent in Europe. In 1928 he suffered a severe illness, which resulted in a serious and permanent reduction of vision. Nevertheless in 1929 he undertook the directorship of the laboratories at the St. Francis

Hospital in Evanston and was there in that capacity when his final illness began in November 1943. He experienced at that time a cerebral



OSCAR T. SCHULTZ, M.D.

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vascular accident which resulted in hemiplegia and total disability lasting until his end in March 1947.

O. T. was a member or fellow of many medical societies—the American Association for the Advancement of Science, the American Association of Pathologists and Bacteriologists, the Society for Experimental Biology and Medicine, the American Medical Association, the Institute of Medicine of Chicago and the Chicago Pathological Society, which he served as president in 1922. During the ten years prior to his illness he became keenly interested in medicolegal matters and was an active member of the Chicago Medico-Legal Society and the Chicago Academy of Criminology. Among his other activities, he was editor of the *Cleveland Medical Journal* from 1910 to 1913 and was visiting pathologist to Columbia Hospital in Milwaukee from 1930 to 1932. He became a member of the editorial board of the ARCHIVES OF PATHOLOGY in 1927 and remained on the board until 1944.

Dr. Schultz's contributions to the literature may be divided into three groups. During his years at Cleveland he contributed papers on syphilis, on topics in the fields of dermatopathology and protozoology and, with Professor Howard, wrote a monograph on tumor cells which was published under a grant from the Rockefeller Institute as one of the series of monographs in medical research. While at Michael Reese Hospital he contributed chiefly to Abt's system of pediatrics—articles on pathologic involvements of the lung and of the kidney and in particular a long section on tumors of infancy and childhood. His third period was from 1929 to 1940, in which he contributed a comprehensive review and critical analysis of the coroner system in the United States, the material concerning which was published in monograph form by the National Research Council, in addition to several shorter papers on the same subject and concerned with limited phases published in different medical journals.

Essentially O. T. was not a research investigator, but those of us who worked with him and knew him well appreciated his great capacity as a pathologist and his rather remarkable memory of medical literature. He was a teacher not for audiences but rather for intimate students and co-workers and, by example, taught the virtues and practices of intelllectual honesty.

A modest man tending to the shy and retiring manner, he was not one to air his burdens and problems, but always appeared to be urbane and at ease. To those few who were intimate with him he was a warm and sympathetic friend. I regarded him as a highly civilized person, entirely free of pettiness and prejudice. He was one of that all too rare kind not affected by current popular hysterias, not given to hasty, impulsive expressions. He was good company; over a lunch table or in intimate evening gatherings he contributed to lusty discussion in many fields. He had a genuine fondness for music and a lively

appreciation for "good living." O. T. served his profession with great devotion and conscientiousness. He was a source of advice and assistance, given without stint and in full measure of his capacities. All who worked with him or under his direction will remember him with affection—for his kindliness, courtesy and respect for human dignity. Surgeons who witnessed his great skill with tissues will remember him for his remarkable accuracy of diagnosis, maintained in spite of handicaps of vision and health.

NORBERT ENZER, M.D.